

1 agggagagagc agtgaccatg aaggctgtgc tgcctgccc gtagatggca  
51 ggcttggccc tgcagccagg cactgcccgt ctgtgctact cctgcaaagc  
101 ccaggtgagc aacgaggact gctgcagggt ggagaactgc acccagctgg  
151 gggagcagtg ctggaccgct cgcattccgc cagtggcct cctgaccgtc  
201 atcagcaaag gctgcagctt gaactgcgtg gatgactcac aggactacta  
251 cgtgggcaag aagaacatca cgtgctgtga caccgacttg tgcaacgcca  
301 gcggggccca tgcctgcag ccggctgccc ccattcctgc gctgctccct  
351 gcactcggcc tgcctgctctg gggacccggc cagctatagg ctctgggggg  
401 ccccgtgca gccacactg ggtgtgtgtc ccaggcctt tgtgccactc  
451 ctacagaac ctggcccagt gggagcctgt cctggttcct gaggcacatc  
501 ctaacgcaag ttgaccatg tatgtttgca ccccttttc ccnaaccctg  
551 accttccat gggcctttc caggattcct accnggcaga tcagtttag  
601 tganacanat ccgctgcag atggccctc caacnnttt tgttntgt  
651 tccatggccc agcattttc acccttaacc ctgtgttcag gcacttttc  
701 ccccaggaag ccttccctgc ccaccccat tatgaattga gccaggttg  
751 gtccgtggtg tccccgcac ccagcagggg acaggcaatc agggaggccc  
801 agtaagggct gtagatgagt ggactgagta gacttgagg acaagagtg  
851 acgtgagttc ctgggagttt ccaggagatgg ggcctggagg cctggaggaa  
901 ggggccaggc ctacattg tggggnccc gaatggcagc ctgagcaccg  
951 cgtaggccct taataaacac ctgtggata agcc222222 22222222

FIGURE 1A

[illegible]

**FIGURE 1B**

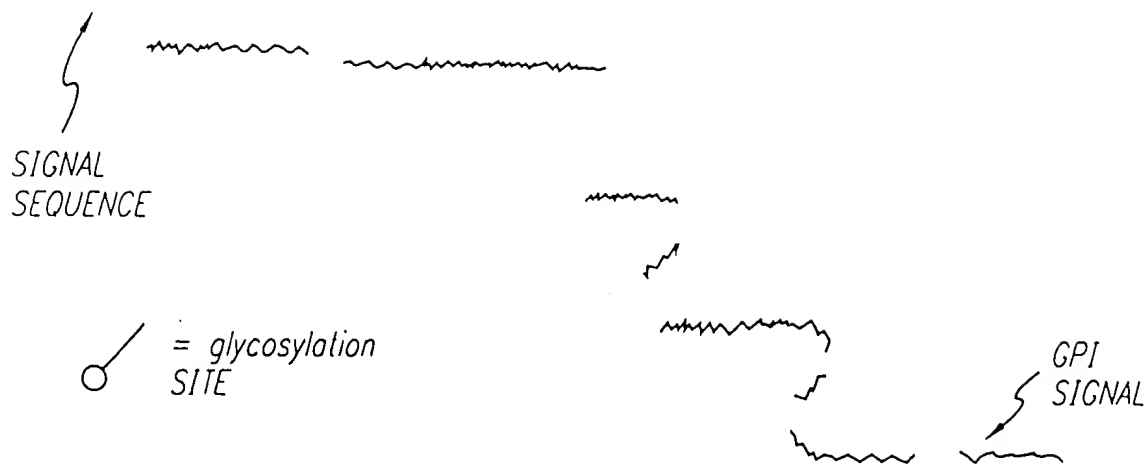
Case	Year	Country	Population	Age	Gender	Occupation	Education	Income	Health	Family	Social	Environment	Other
1	1998	USA	250,000,000	45	Male	Teacher	High School	\$30,000	Good	2 Children	Married	Suburban	None
2	2001	USA	270,000,000	52	Female	Nurse	College	\$40,000	Fair	1 Child	Married	Urban	Smoking
3	2005	USA	290,000,000	60	Male	Engineer	University	\$60,000	Excellent	3 Children	Married	Suburban	None
4	2008	USA	310,000,000	68	Female	Retired	High School	\$20,000	Poor	4 Children	Married	Urban	Smoking
5	2012	USA	330,000,000	75	Male	Unemployed	High School	\$10,000	Very Poor	5 Children	Married	Urban	Smoking

1927 1928 1929 1930 1931 1932 1933 1934 1935 1936 1937 1938 1939 1940 1941 1942 1943 1944 1945 1946 1947 1948 1949 1950 1951 1952 1953 1954 1955 1956 1957 1958 1959 1960 1961 1962 1963 1964 1965 1966 1967 1968 1969 1970 1971 1972 1973 1974 1975 1976 1977 1978 1979 1980 1981 1982 1983 1984 1985 1986 1987 1988 1989 1990 1991 1992 1993 1994 1995 1996 1997 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015 2016 2017 2018 2019 2020 2021 2022 2023 2024 2025 2026 2027 2028 2029 2030 2031 2032 2033 2034 2035 2036 2037 2038 2039 2040 2041 2042 2043 2044 2045 2046 2047 2048 2049 2050 2051 2052 2053 2054 2055 2056 2057 2058 2059 2060 2061 2062 2063 2064 2065 2066 2067 2068 2069 2070 2071 2072 2073 2074 2075 2076 2077 2078 2079 2080 2081 2082 2083 2084 2085 2086 2087 2088 2089 2090 2091 2092 2093 2094 2095 2096 2097 2098 2099 2100 2101 2102 2103 2104 2105 2106 2107 2108 2109 2110 2111 2112 2113 2114 2115 2116 2117 2118 2119 2120 2121 2122 2123 2124 2125 2126 2127 2128 2129 2130 2131 2132 2133 2134 2135 2136 2137 2138 2139 2140 2141 2142 2143 2144 2145 2146 2147 2148 2149 2150 2151 2152 2153 2154 2155 2156 2157 2158 2159 2160 2161 2162 2163 2164 2165 2166 2167 2168 2169 2170 2171 2172 2173 2174 2175 2176 2177 2178 2179 2180 2181 2182 2183 2184 2185 2186 2187 2188 2189 2190 2191 2192 2193 2194 2195 2196 2197 2198 2199 2200 2201 2202 2203 2204 2205 2206 2207 2208 2209 2210 2211 2212 2213 2214 2215 2216 2217 2218 2219 2220 2221 2222 2223 2224 2225 2226 2227 2228 2229 2230 2231 2232 2233 2234 2235 2236 2237 2238 2239 2240 2241 2242 2243 2244 2245 2246 2247 2248 2249 2250 2251 2252 2253 2254 2255 2256 2257 2258 2259 2260 2261 2262 2263 2264 2265 2266 2267 2268 2269 2270 2271 2272 2273 2274 2275 2276 2277 2278 2279 2280 2281 2282 2283 2284 2285 2286 2287 2288 2289 2290 2291 2292 2293 2294 2295 2296 2297 2298 2299 2300 2301 2302 2303 2304 2305 2306 2307 2308 2309 2310 2311 2312 2313 2314 2315 2316 2317 2318 2319 2320 2321 2322 2323 2324 2325 2326 2327 2328 2329 2330 2331 2332 2333 2334 2335 2336 2337 2338 2339 2340 2341 2342 2343 2344 2345 2346 2347 2348 2349 2350 2351 2352 2353 2354 2355 2356 2357 2358 2359 2360 2361 2362 2363 2364 2365 2366 2367 2368 2369 2370 2371 2372 2373 2374 2375 2376 2377 2378 2379 2380 2381 2382 2383 2384 2385 2386 2387 2388 2389 2390 2391 2392 2393 2394 2395 2396 2397 2398 2399 2400 2401 2402 2403 2404 2405 2406 2407 2408 2409 2410 2411 2412 2413 2414 2415 2416 2417 2418 2419 2420 2421 2422 2423 2424 2425 2426 2427 2428 2429 2430 2431 2432 2433 2434 2435 2436 2437 2438 2439 2440 2441 2442 2443 2444 2445 2446 2447 2448 2449 2450 2451 2452 2453 2454 2455 2456 2457 2458 2459 2460 2461 2462 2463 2464 2465 2466 2467 2468 2469 2470 2471 2472 2473 2474 2475 2476 2477 2478 2479 2480 2481 2482 2483 2484 2485 2486 2487 2488 2489 2490 2491 2492 2493 2494 2495 2496 2497 2498 2499 2500 2501 2502 2503 2504 2505 2506 2507 2508 2509 2510 2511 2512 2513 2514 2515 2516 2517 2518 2519 2520 2521 2522 2523 2524 2525 2526 2527 2528 2529 2530 2531 2532 2533 2534 2535 2536 2537 2538 2539 2540 2541 2542 2543 2544 2545 2546 2547 2548 2549 2550 2551 2552 2553 2554 2555 2556 2557 2558 2559 2560 2561 2562 2563 2564 2565 2566 2567 2568 2569 2570 2571 2572 2573 2574 2575 2576 2577 2578 2579 2580 2581 2582 2583 2584 2585 2586 2587 2588 2589 2590 2591 2592 2593 2594 2595 2596 2597 2598 2599 2600 2601 2602 2603 2604 2605 2606 2607 2608 2609 2610 2611 2612 2613 2614 2615 2616 2617 2618 2619 2620 2621 2622 2623 2624 2625 2626 2627 2628 2629 2630 2631 2632 2633 2634 2635 2636 2637 2638 2639 2640 2641 2642 2643 2644 2645 2646 2647 2648 2649 2650 2651 2652 2653 2654 2655 2656 2657 2658 2659 2660 2661 2662 2663 2664 2665 2666 2667 2668 2669 2670 2671 2672 2673 2674 2675 2676 2677 2678 2679 2680 2681 2682 2683 2684 2685 2686 2687 2688 2689 2690 2691 2692 2693 2694 2695 2696 2697 2698 2699 2700 2701 2702 2703 2704 2705 2706 2707 2708 2709 2710 2711 2712 2713 2714 2715 2716 2717 2718 2719 2720 2721 2722 2723 2724 2725 2726 2727 2728 2729 2730 2731 2732 2733 2734 2735 2736 2737 2738 2739 2740 2741 2742 2743 2744 2

### FIGURE 3

The figure displays seven distinct signal waveforms arranged vertically. From top to bottom: 1) A flat line with minor noise. 2) A smooth, periodic sine wave. 3) A noisy signal with irregular peaks and valleys. 4) A square wave alternating between two levels. 5) A complex digital signal with multiple levels and sharp transitions. 6) A step function that changes its value at specific points. 7) A series of narrow, periodic pulses.

FIGURE 4



**FIGURE 5**

Western BSA  
 Superoxide 80% AB  
 Normal tissue  
 1hr exp

1G8  
 1:100

prostate (H&E)  
 prostate (Baker)  
 prostate (dck)  
 Bladder (H&E)  
 Bladder (dck)  
 Bladder (Ab)  
 Kidney (NABG)  
 Kidney (WU2)  
 Testis  
 Sm. Intest.

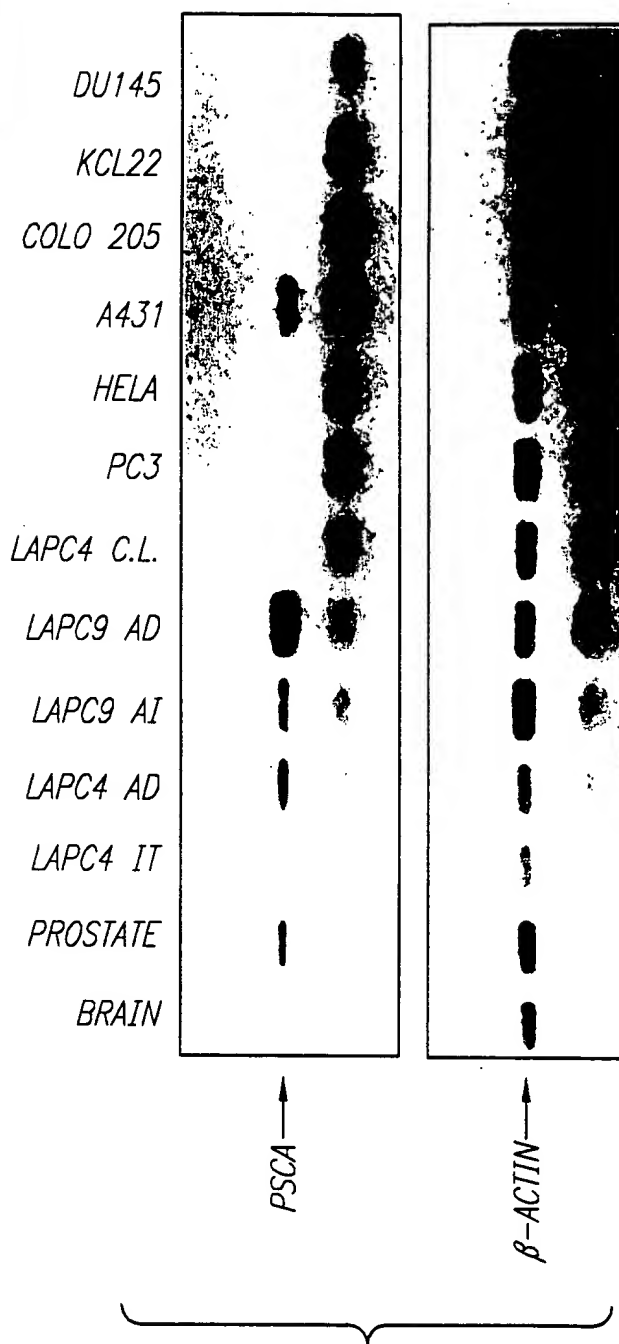
LA PC9

FIGURE 6

7A

+CONTROL  
TONSILS  
THYMUS  
SPLEEN  
LEUKOCYTES  
LYMPH NODE  
FETAL LIVER  
BONE MARROW  
THYMUS  
TESTIS  
SPLEEN  
SM. INTESTINE  
PROSTATE  
LEUKOCYTES  
OVARY  
COLON  
SKELETAL MUSCLE  
PLACENTA  
PANCREAS  
LUNG  
LIVER  
KIDNEY  
HEART  
BRAIN


PSCA





669203362560

Legend:  untranslated region of PSCA

 translated region of PSCA

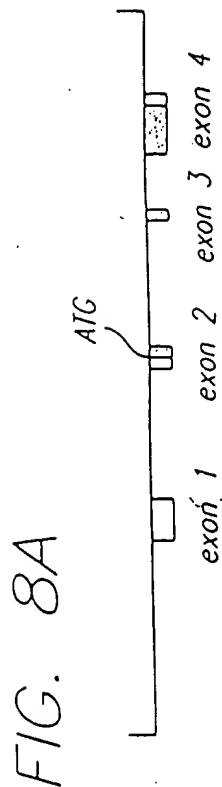


FIG. 8B

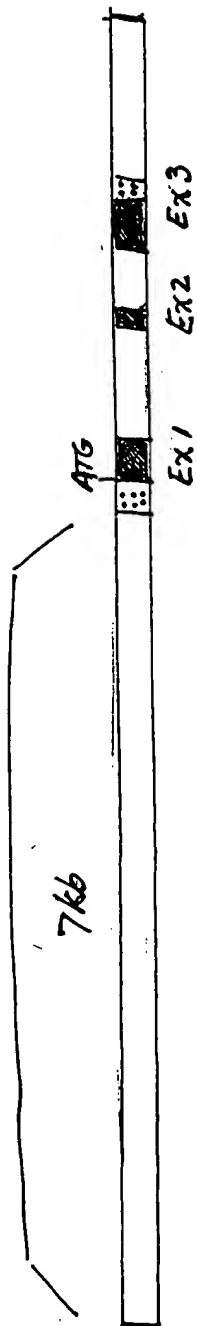
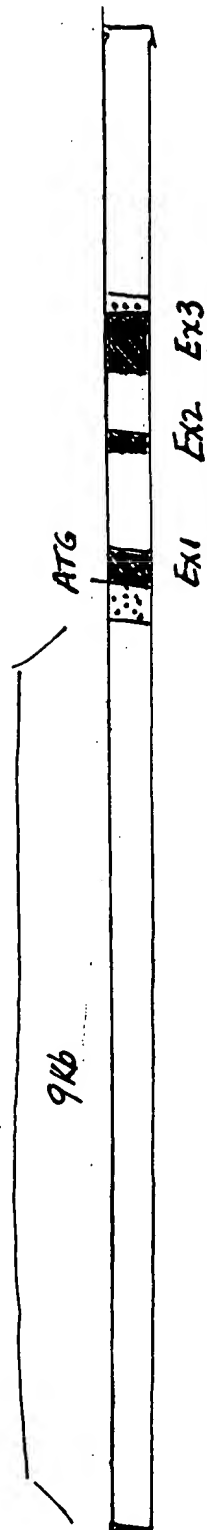


FIG. 8C



transcribed PSCA

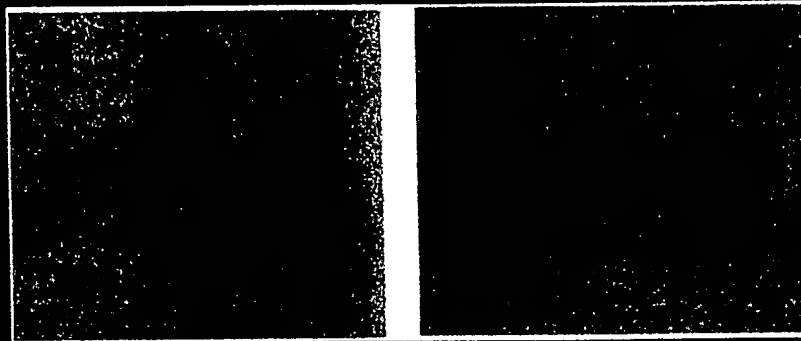
FIGURE 8

transcribed PSCA

# PSCA / PSA Expression in Benign Prostate vs. Prostate Cancer Xenograft

Benign  
LAPC-4 AD  
LAPC-4 AI

Benign  
LAPC-4 AD  
LAPC-4 AI



PSCA

PSA

FIGURE 9A

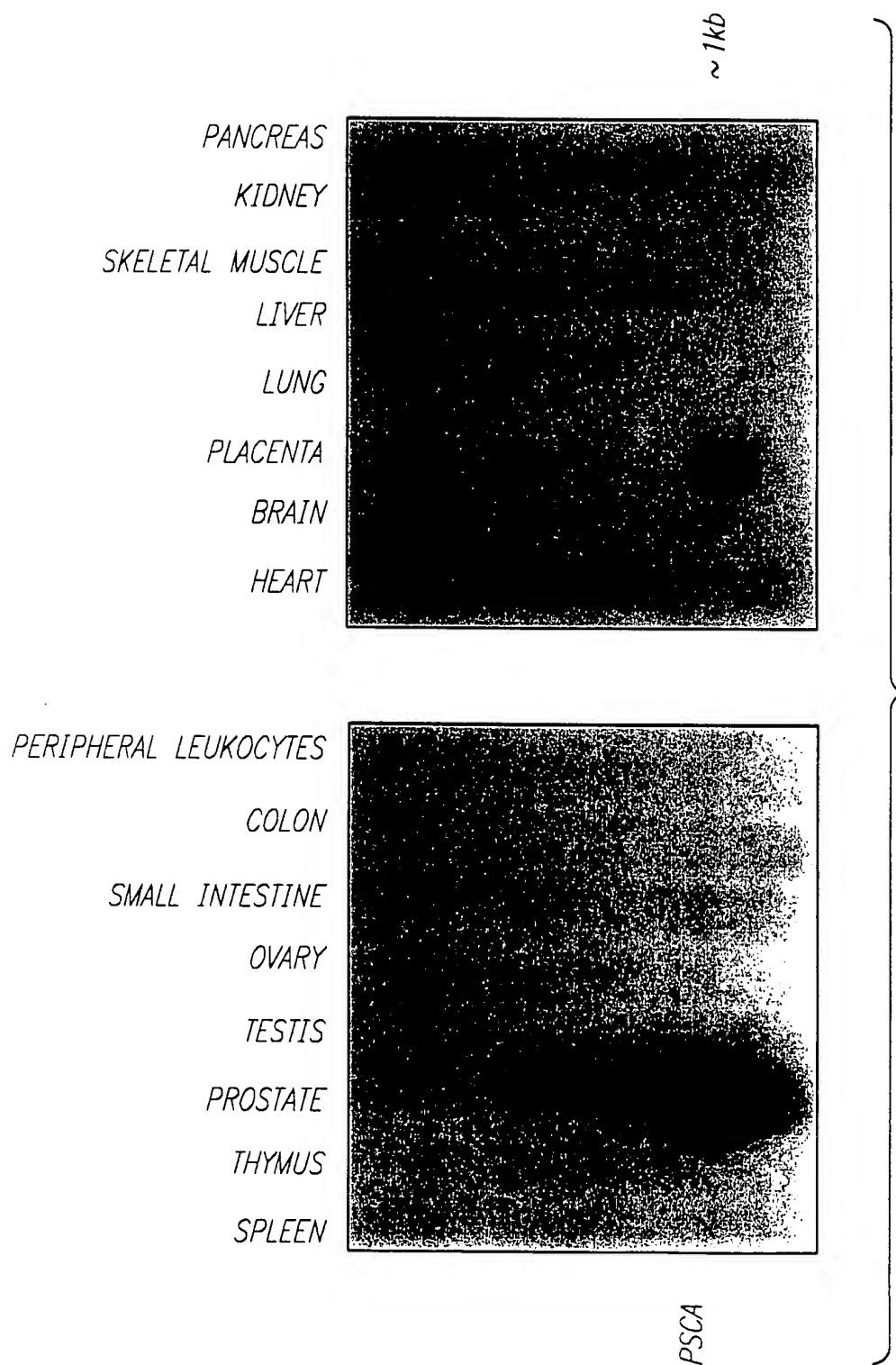
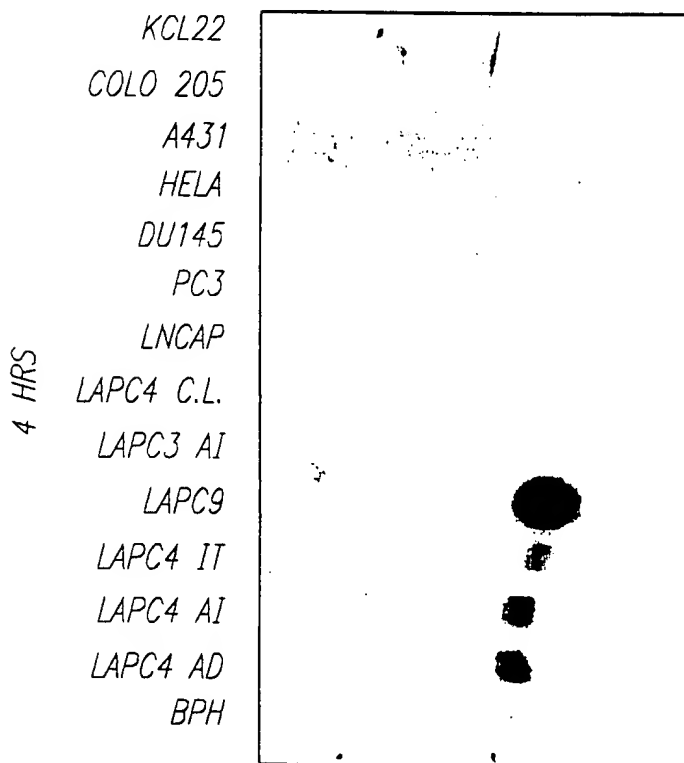
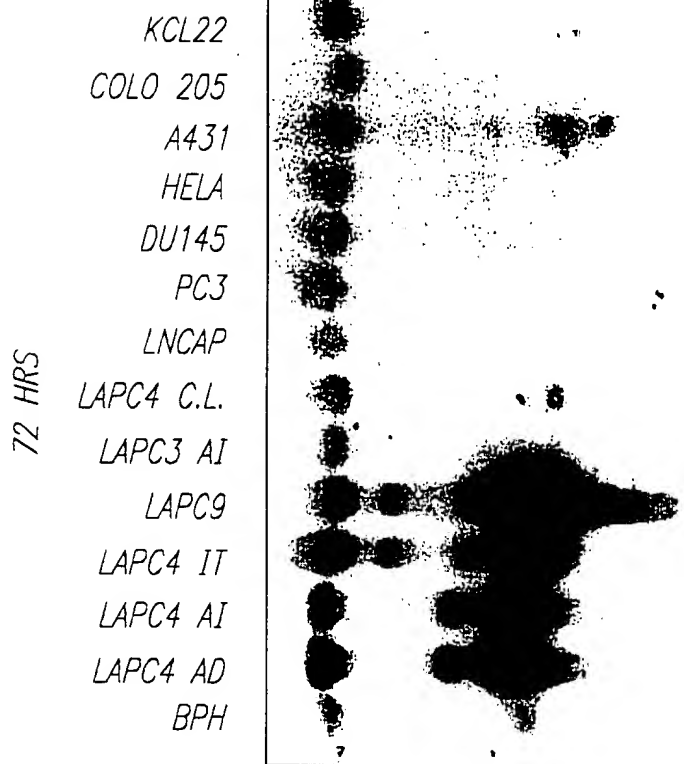


FIG. 9B

660260 386560



PSCA

FIG. 10-1

66020" 32E65E60

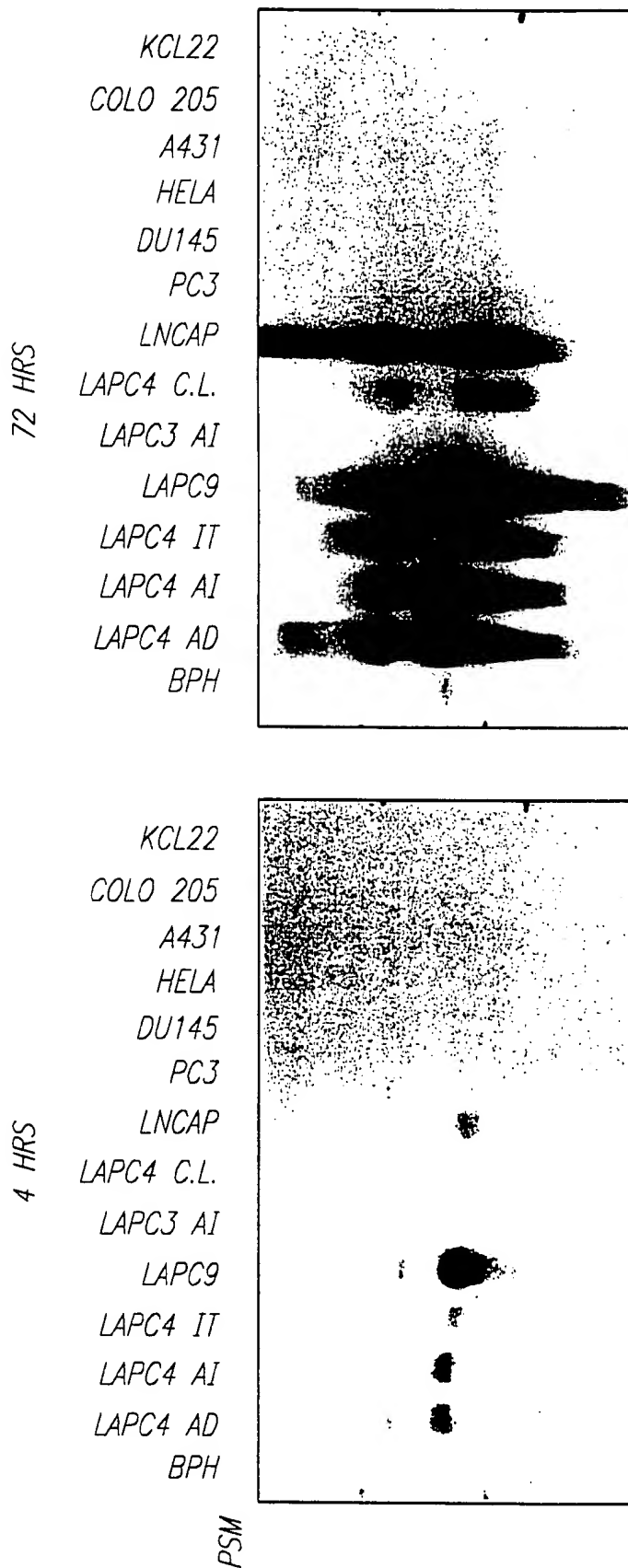
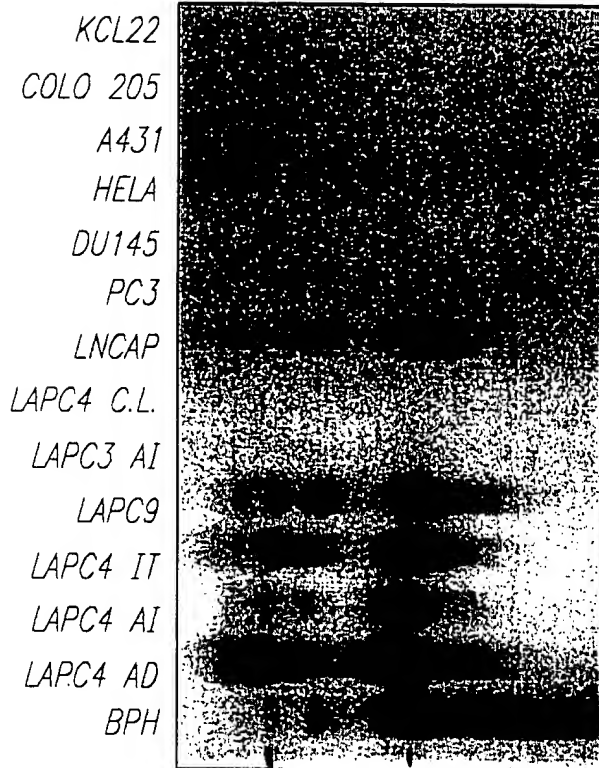


FIG. 10-2

66060" 336560

72 HRS



4 HRS

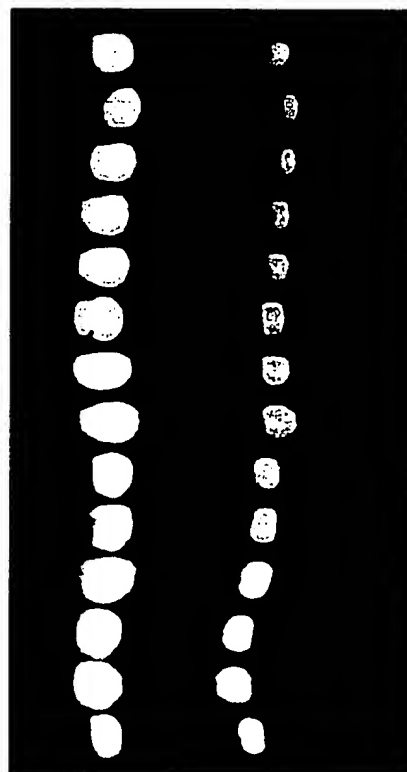
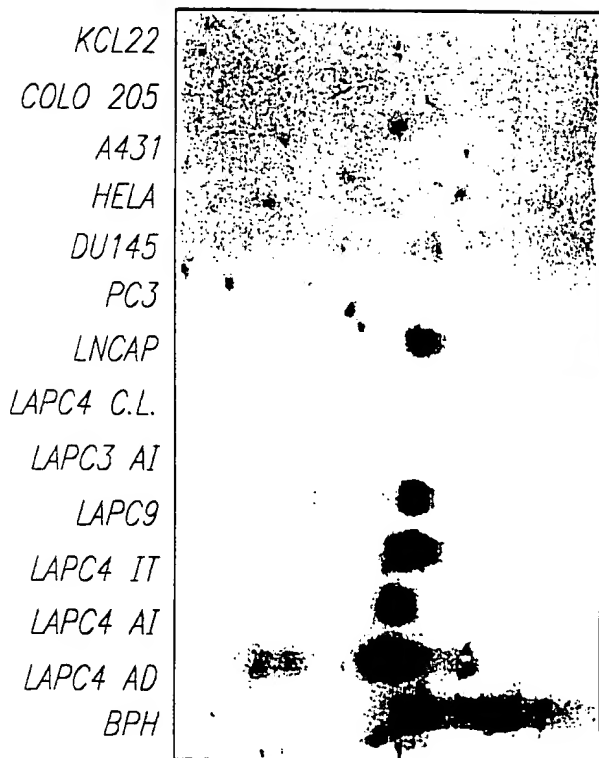


FIG. 10-3

FIG. 11A



FIG. 11B

550220" 9265660

60000' 9266560

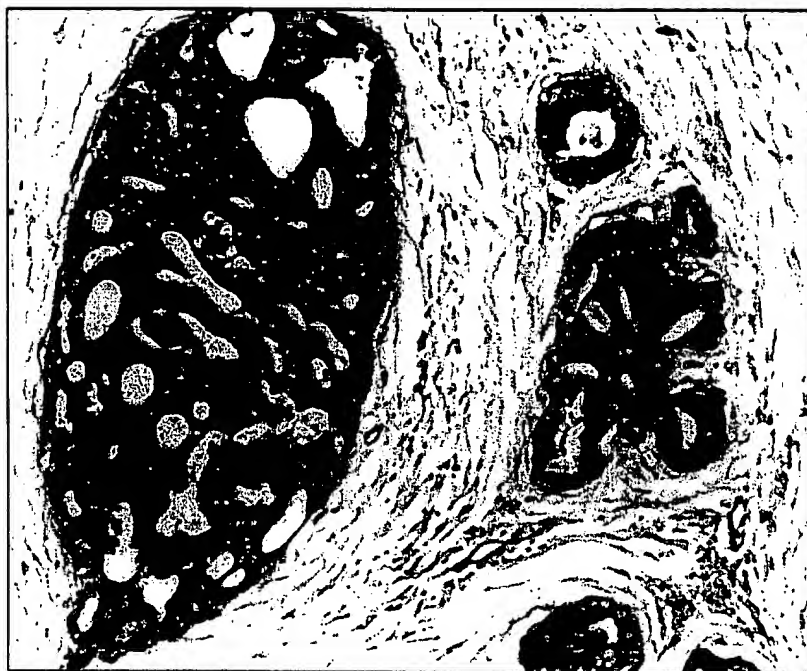


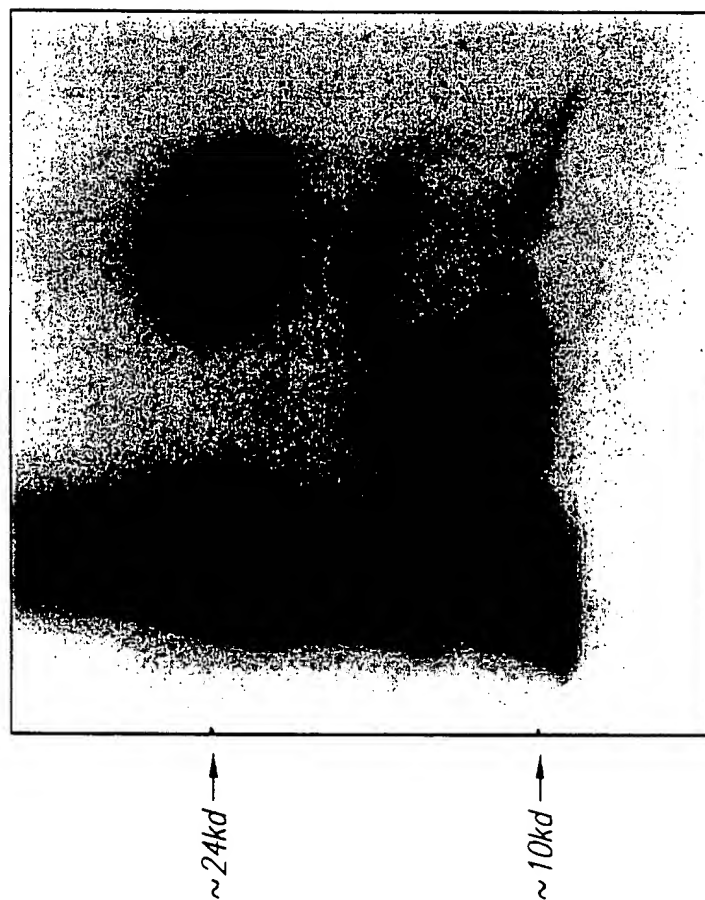
FIG. 11C



660620" 32263260

FIG. 12A

CONTROL  
N GLYCOSIDASE F  
O GLYCOSIDASE



CELL ASSOCIATED

SECRETED

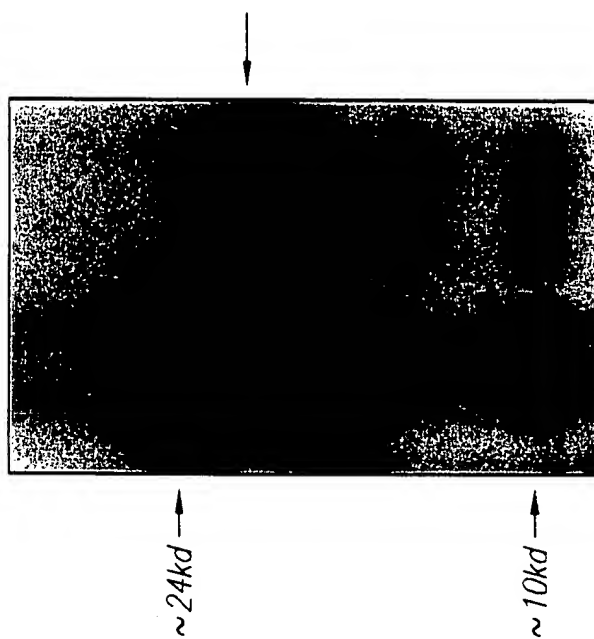


FIG. 12B

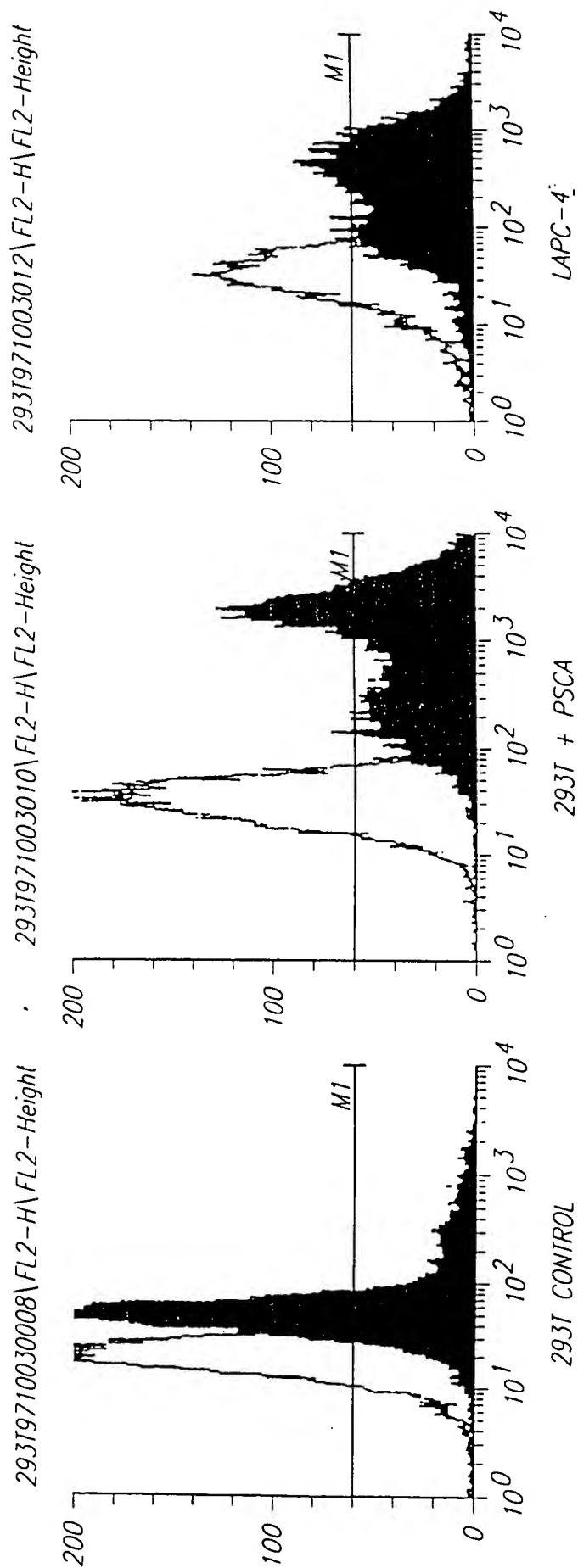
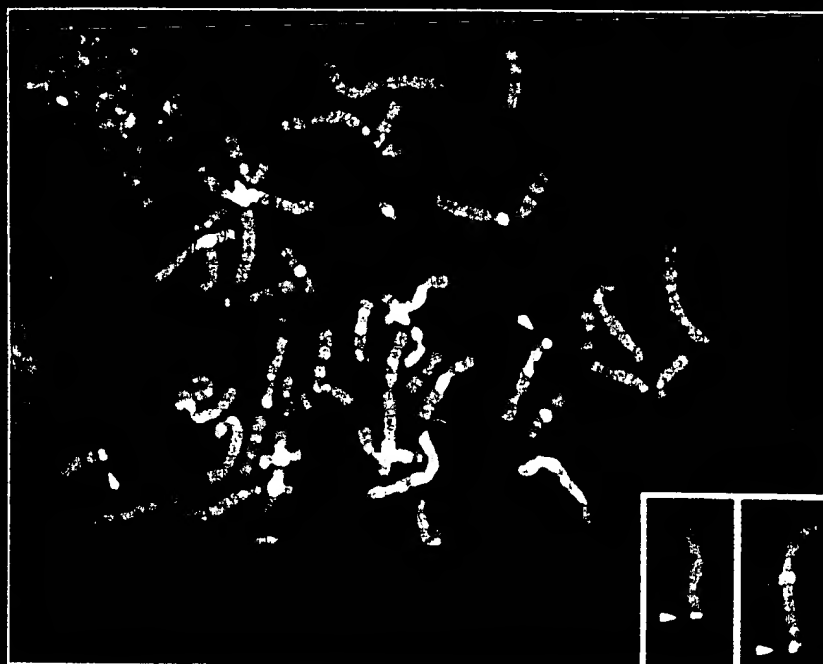


FIGURE 12C

## PSCA Maps to Chromosome 8q24.2



Fluorescent  
in Situ Hybridization  
Analysis of PSCA

FIGURE 13

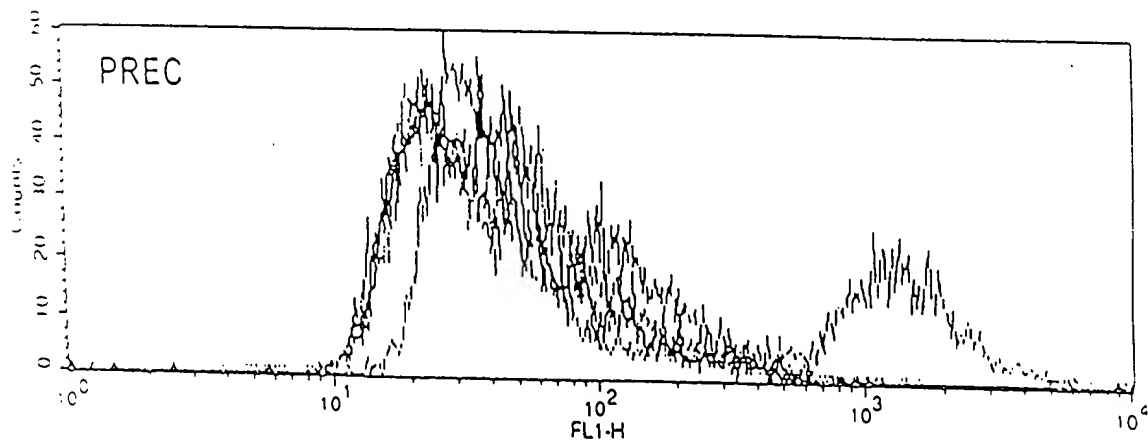
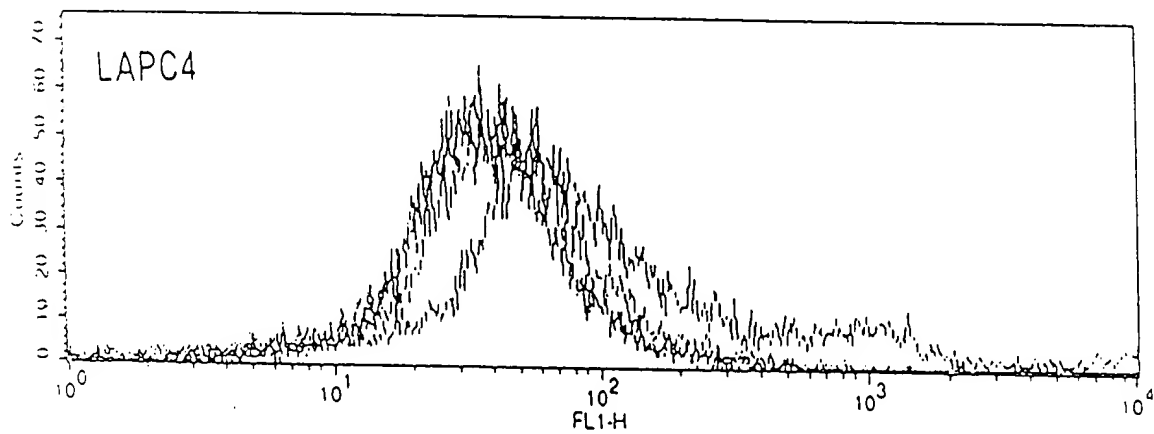
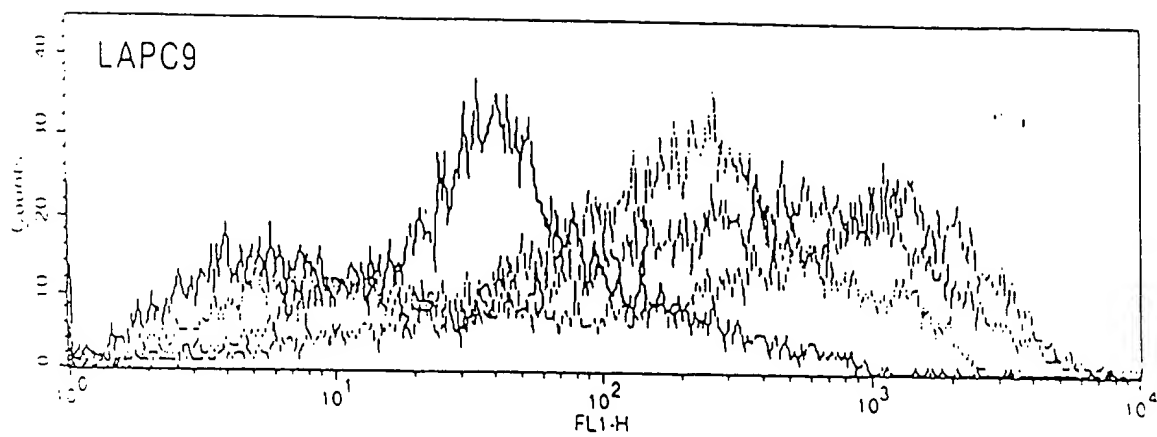


FIGURE 14

**A**

mAb	Isotype	FL (18-98)	N (2-50)	M (46-109)	C (85-123)
1G8	IgG1 k	2.039	0.007	0.628	0.000
2H9	IgG1 k	1.318	0.863	0.032	0.021
3C5	IgG2a k	2.893	1.965	0.016	0.005
3E6	IgG3 k	0.328	0.024	0.069	0.370
4A10	IgG2a k	2.039	1.315	0.000	0.014
2A2	IgG2a k	1.366	0.733	0.010	0.003
3G3	IgG2a k	2.805	1.731	0.004	0.000

**B**

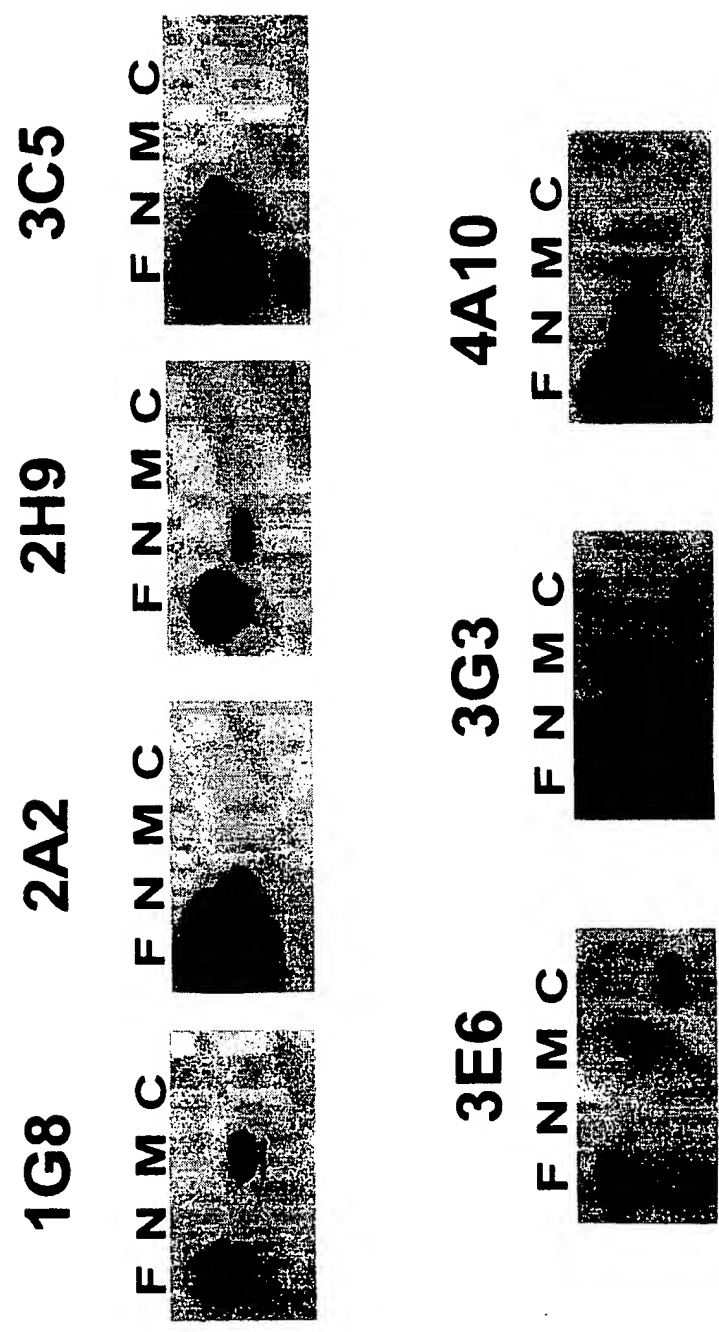


FIGURE 15

# Prostate Stem Cell Antigen (PSCA) is a GPI-anchored Protein

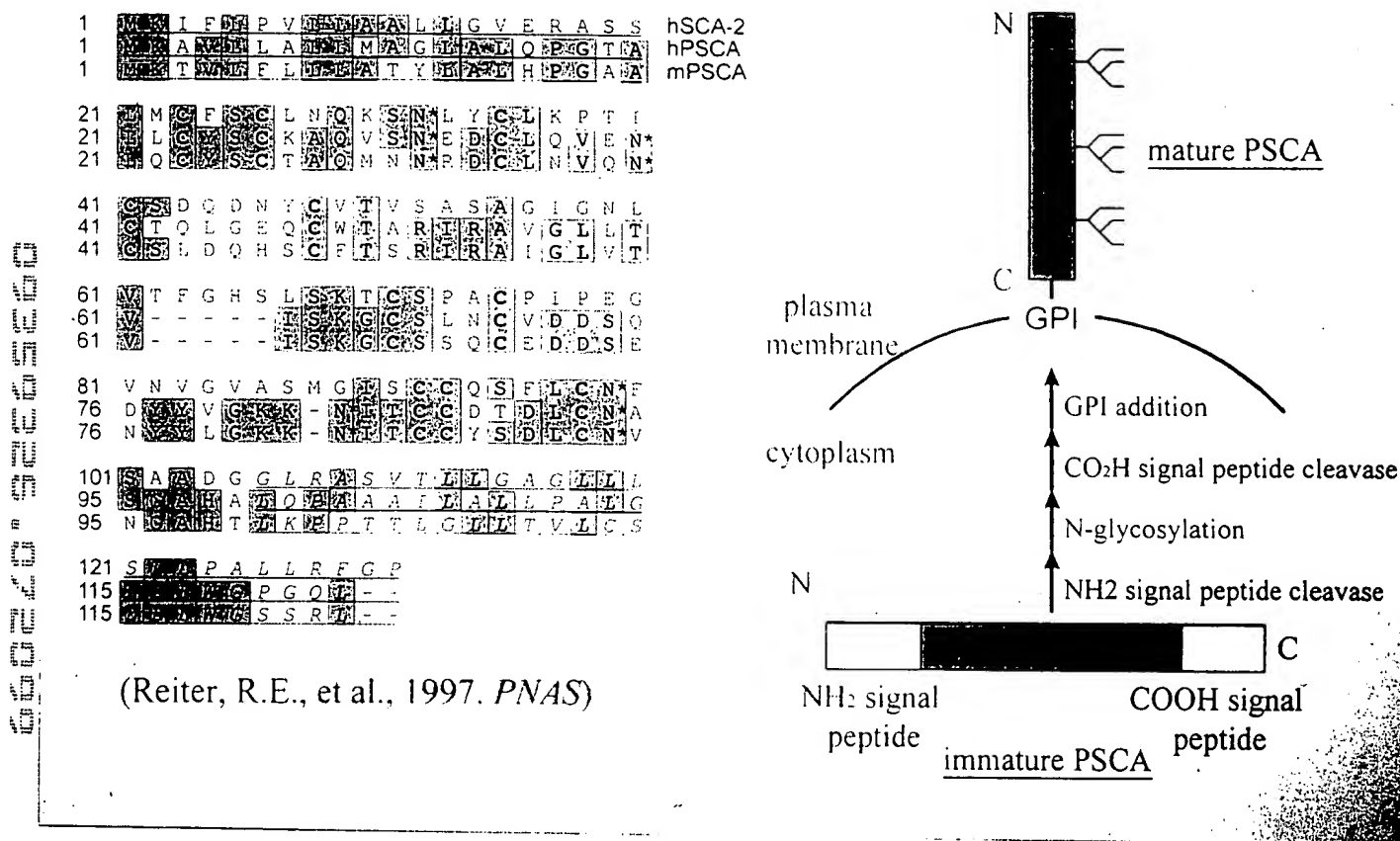
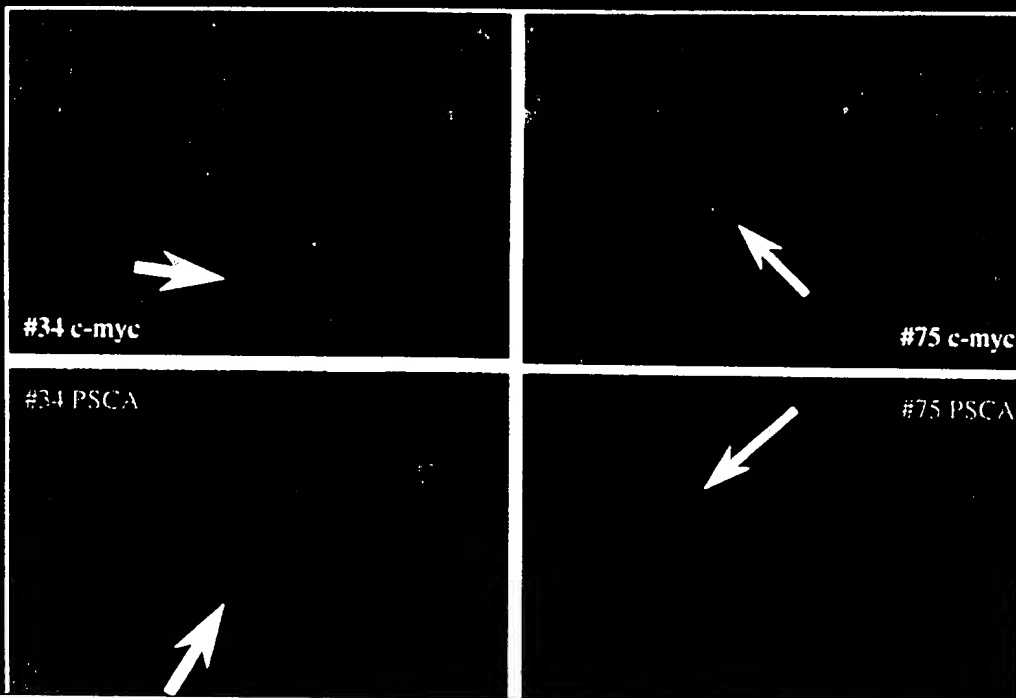


FIGURE 16

# FISH Analysis of PSCA and c-myc in Prostate Cancer

Gain Chromosome 8

Amplification



*R. Jenkins*

FIGURE 17

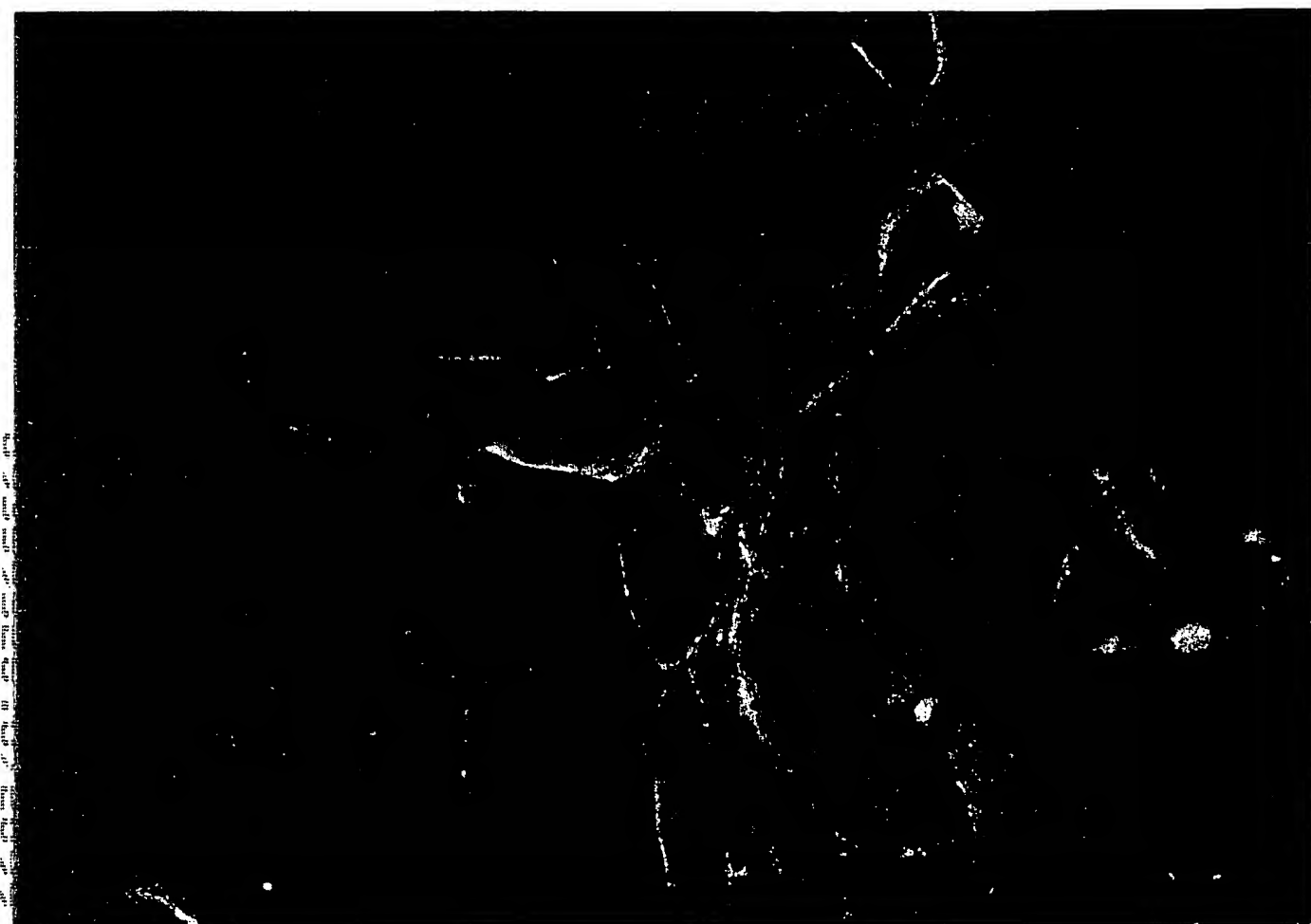


FIGURE 18



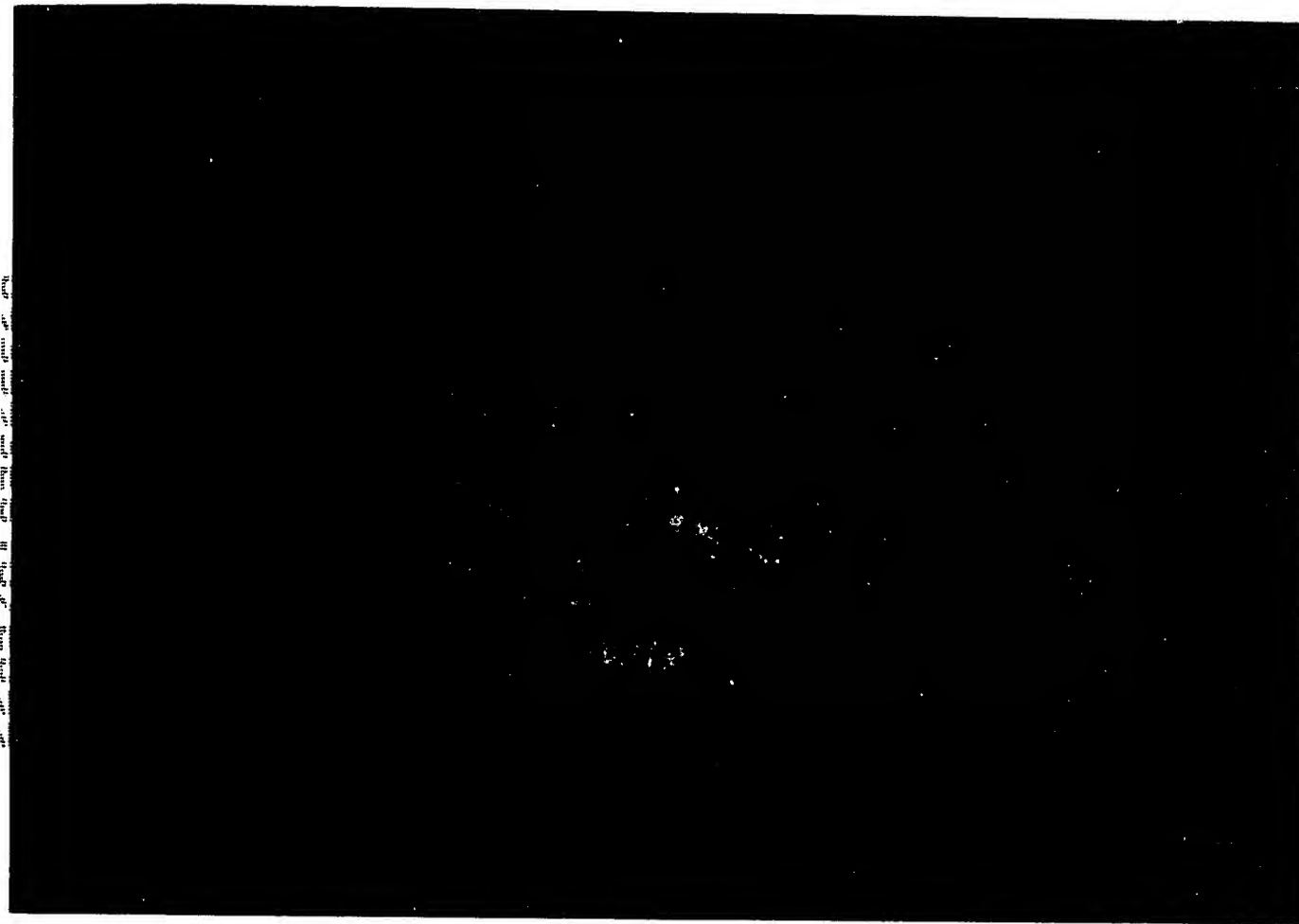


FIGURE 19

60220 3265260



FIGURE 20

## PSCA Immunostaining of Primary Tumors

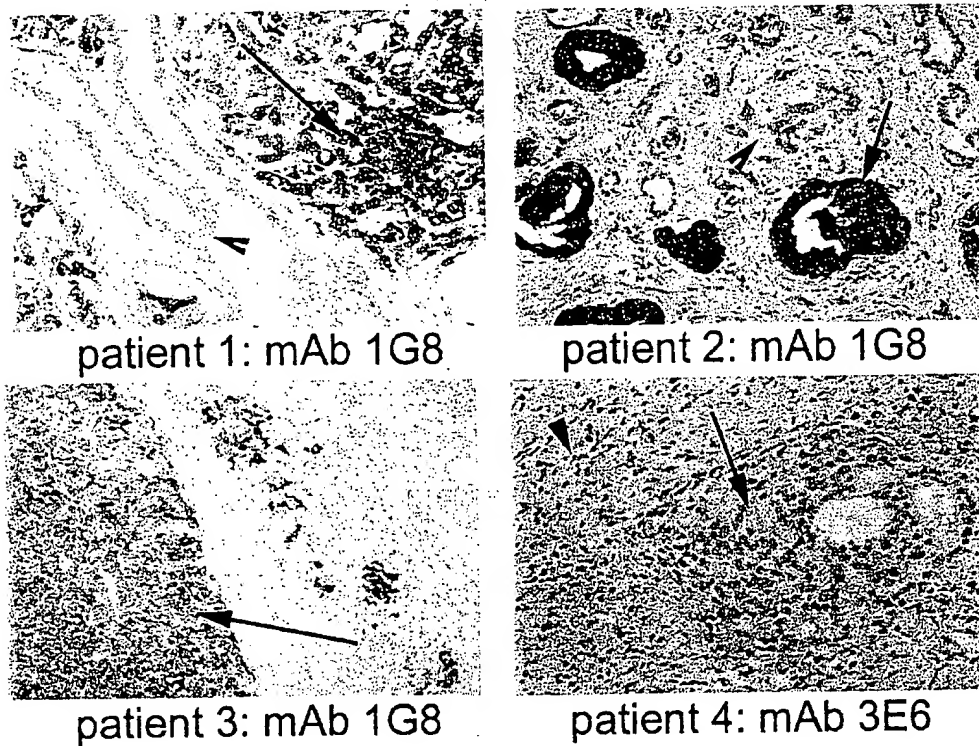


FIGURE 21



FIGURE 22

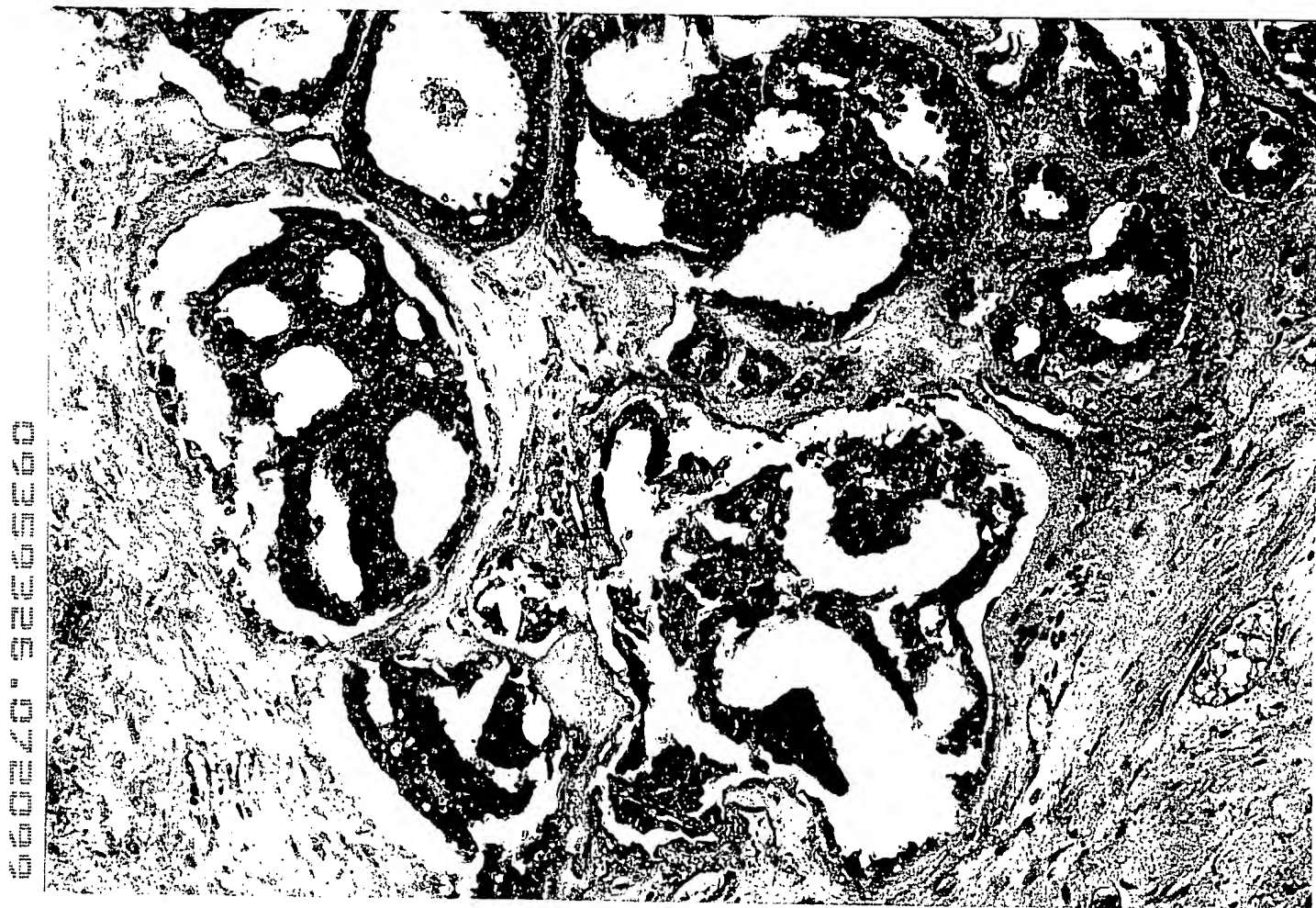


FIGURE 23

660220" 92265260

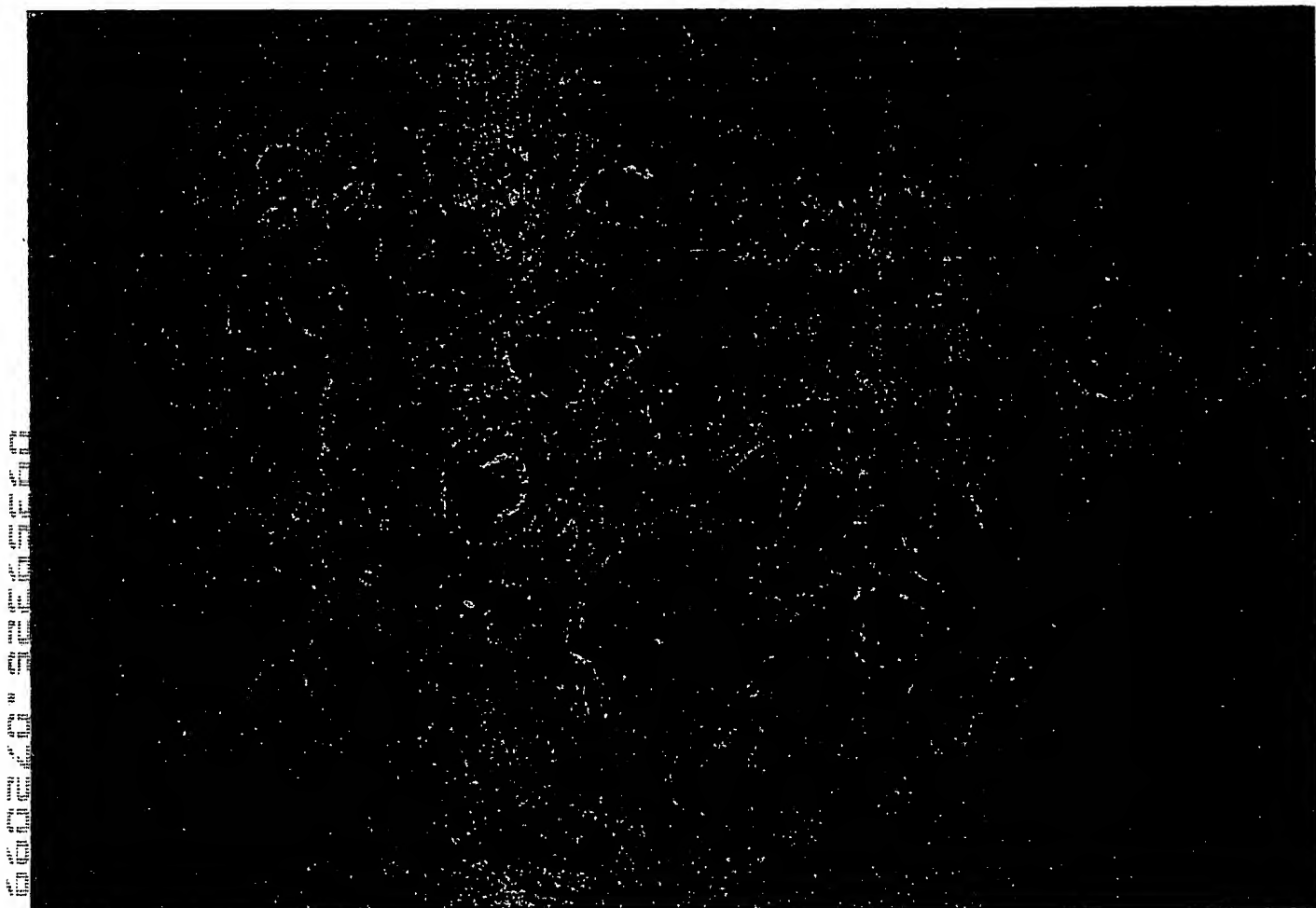


**FIGURE 24**



A hand-drawn anatomical diagram of the human urinary system. The diagram shows the kidneys at the top, connected by the ureters to the bladder in the center. The ureters are labeled 'Ureter' and the bladder is labeled 'Bladder'. The kidneys are labeled 'Kidney'.

FIGURE 25



**FIGURE 26**



66020 36360

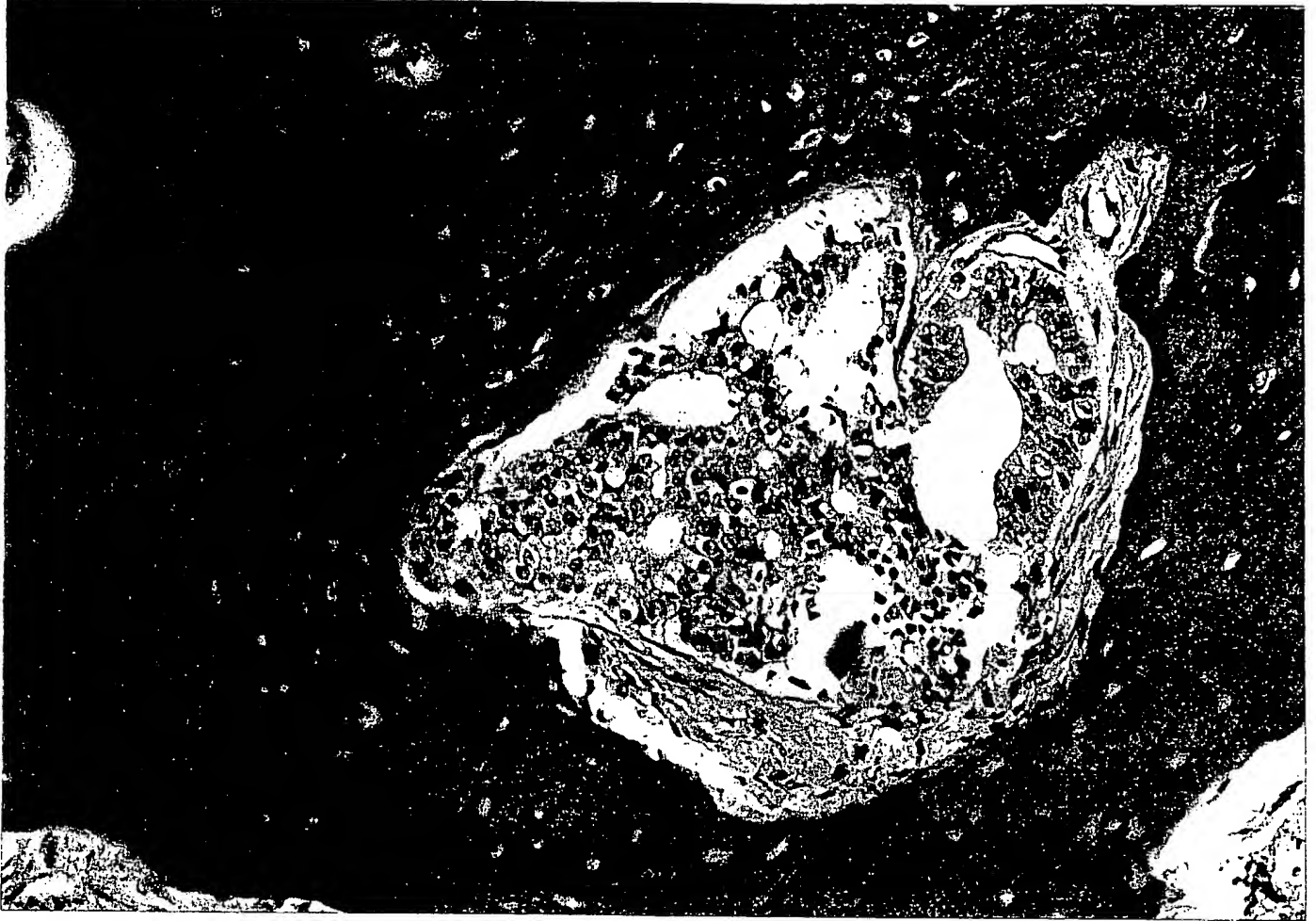
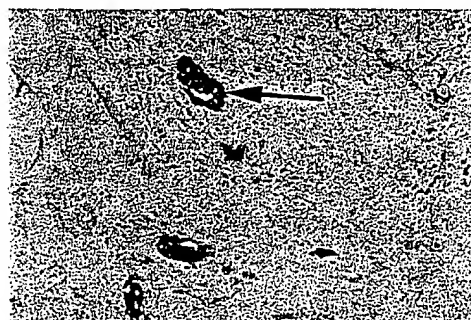
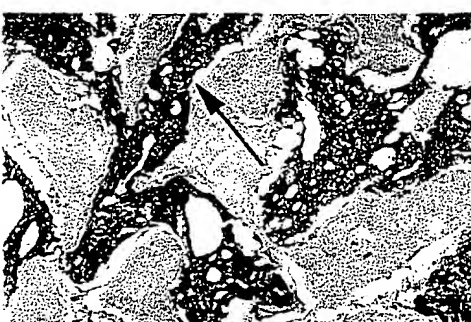


FIGURE 27

## PSCA Immunostaining of Bony Metastases



Patient 5: H and E  
and mAb 1G8



Patient 4: H and E  
and mAb 3E6

FIGURE 28

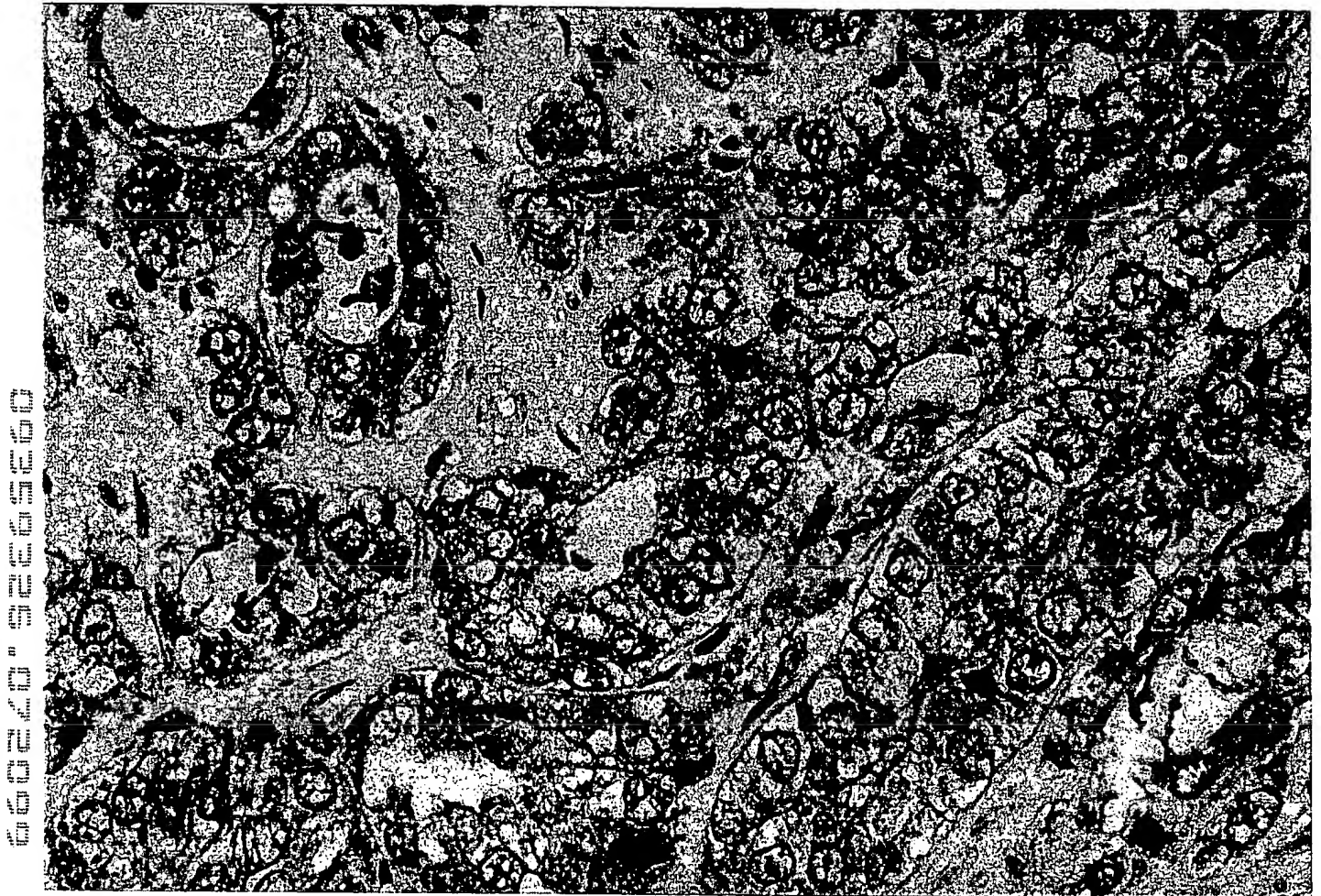
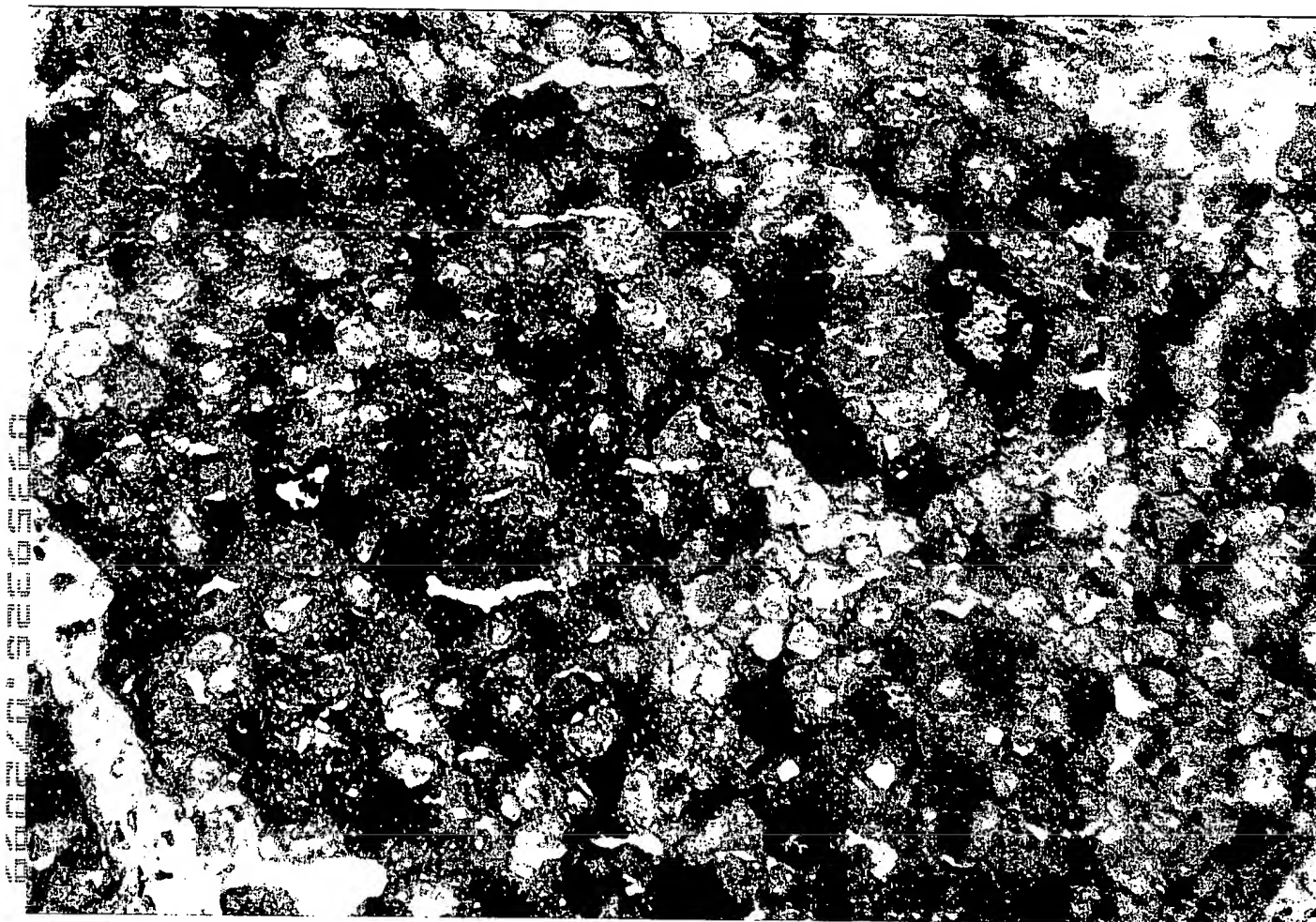


FIGURE 29



**FIGURE 30**

660220" 22655260

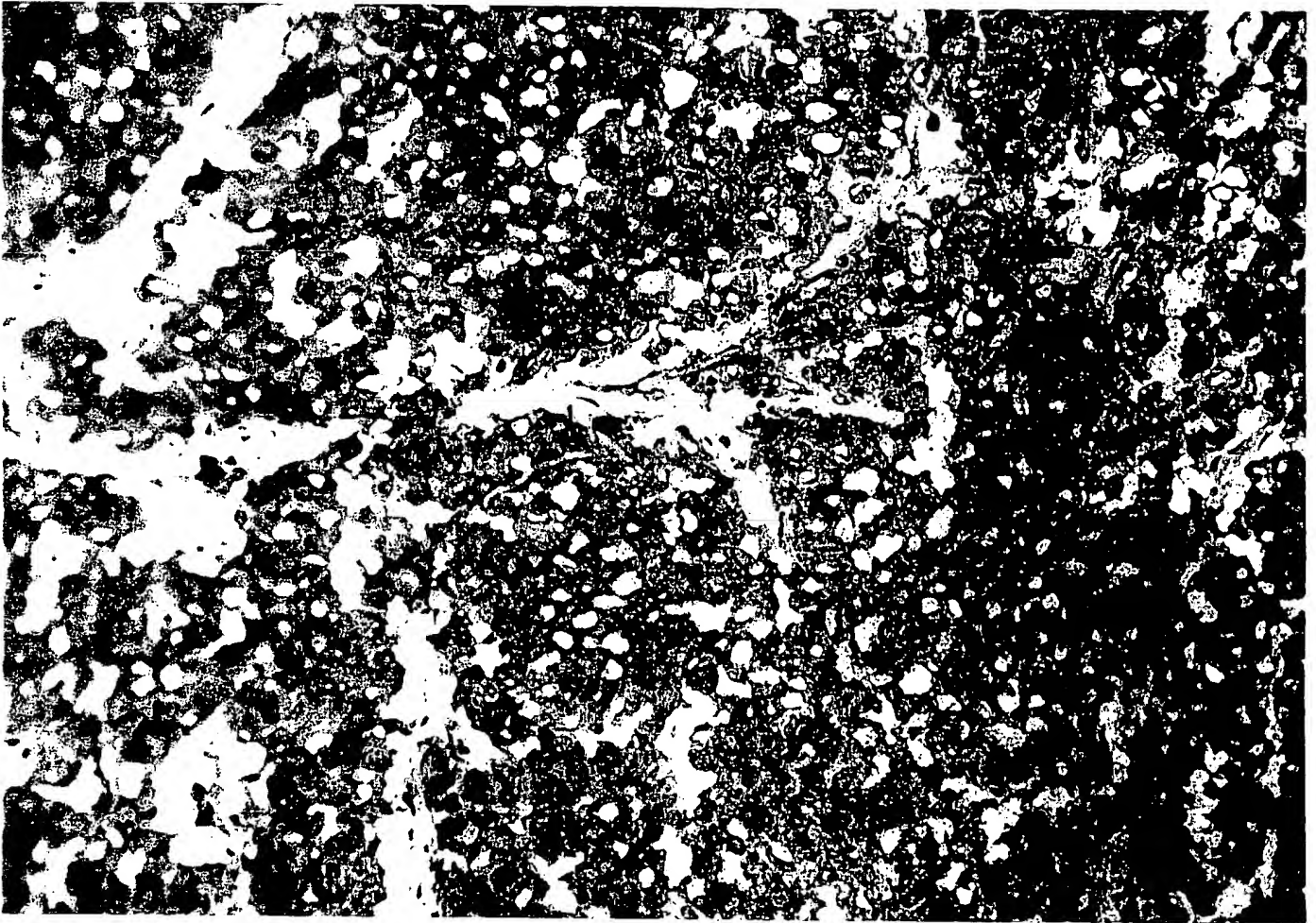
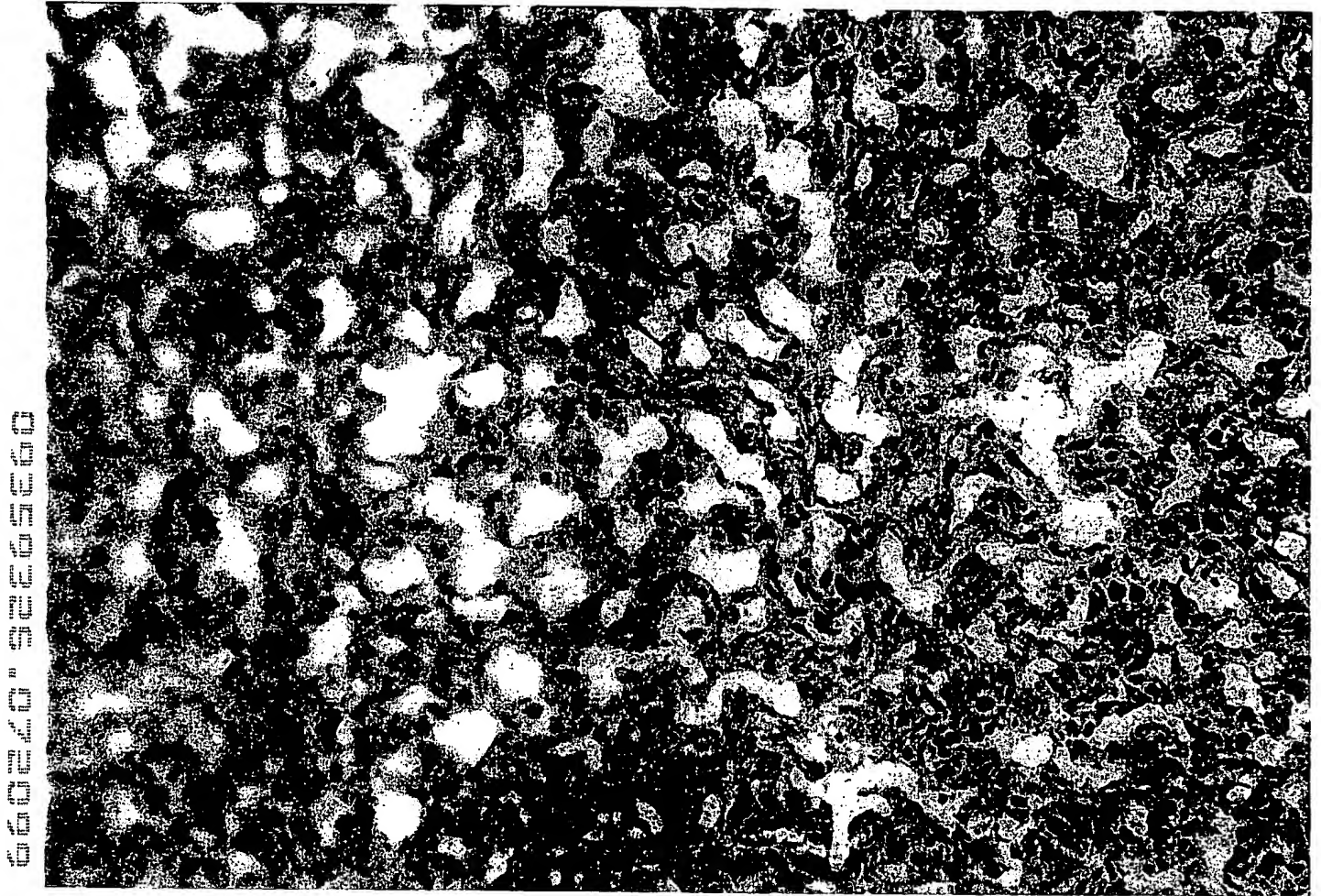


FIGURE 31





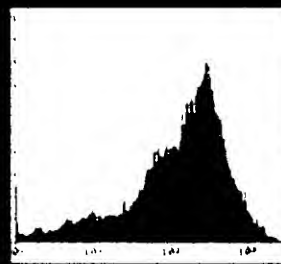
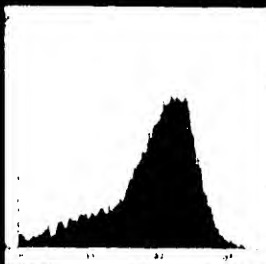
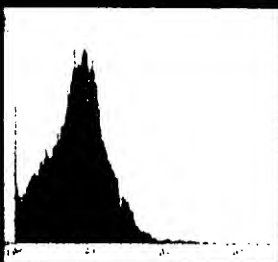
**FIGURE 32**

# PSCA Expression in LAPC-9 Xenograft by FACS

Secondary Antibody

1G8

2H9



4A10

3C5

3E6

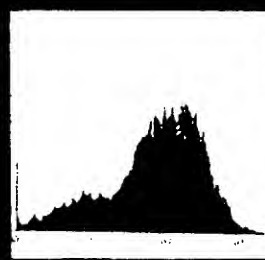
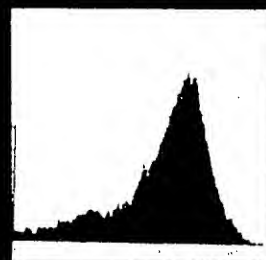
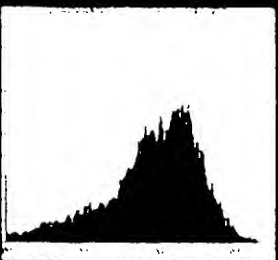


FIGURE 33

660260 336560

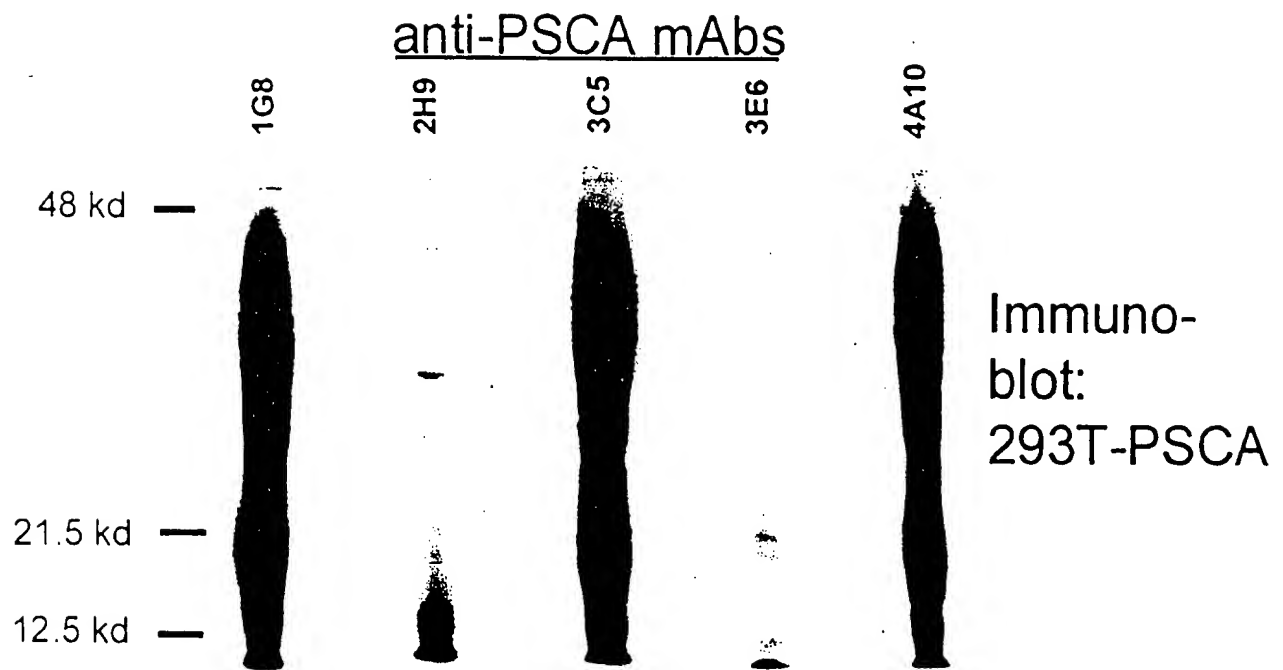


FIGURE 34



## Immunofluorescent Staining of LNCaP-PSCA Cells

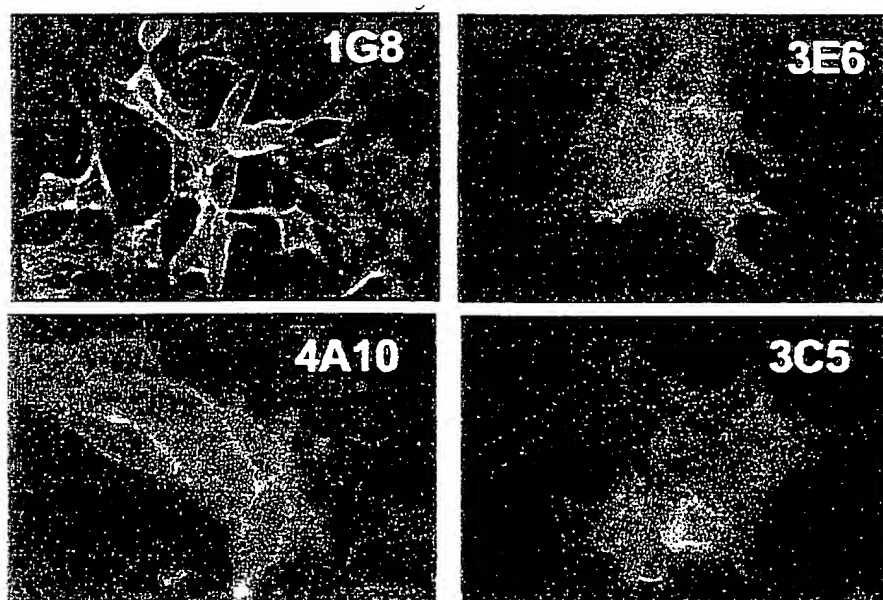


FIGURE 35

660207 300300Z



FIGURE 36

66020" 32265200

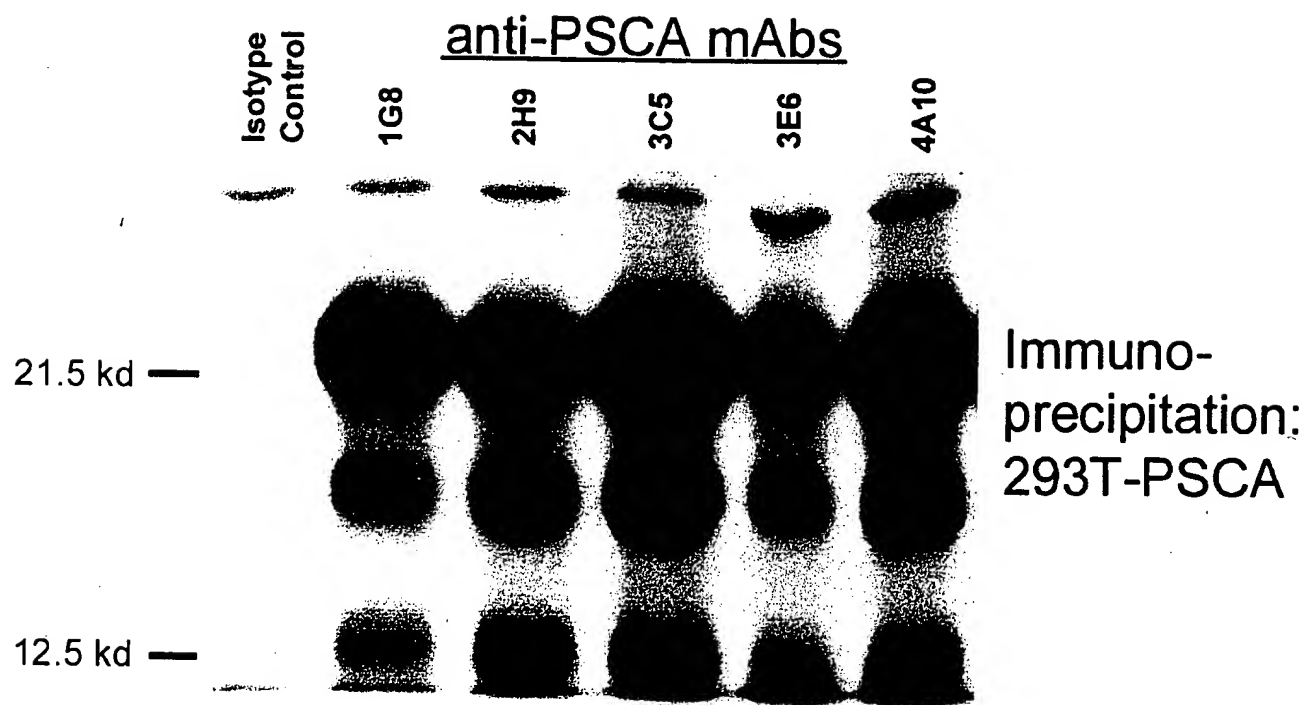
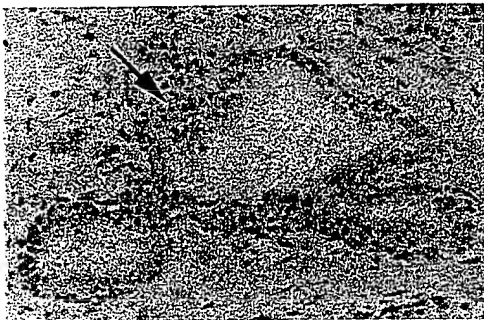


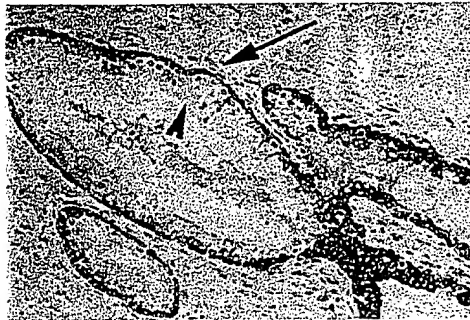
FIGURE 37

## Immunohistochemical Staining of Normal Prostate

Normal: Isotype Control



Normal: PSCA mAb 3E6



Normal: PSCA mAb 1G8



Atrophy: PSCA mAb 2H9

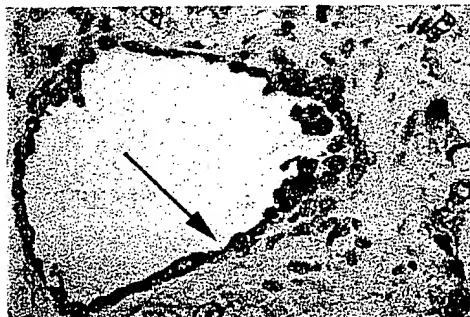
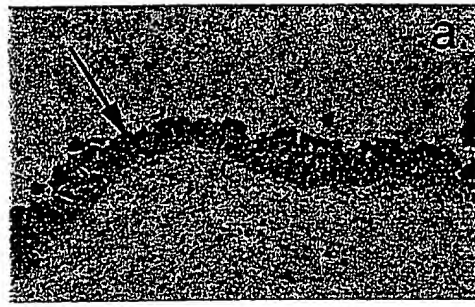


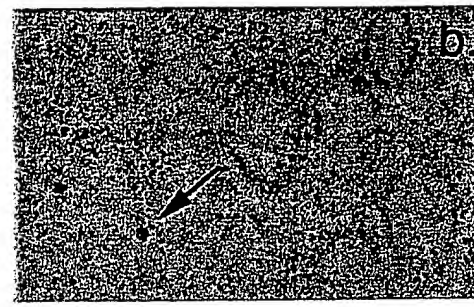
FIGURE 38

660220" 92E69E60

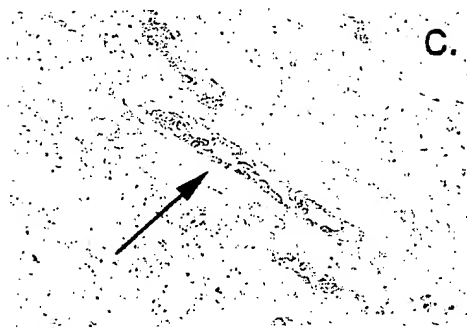
A.



Bladder: 1G8



Colon: 1G8



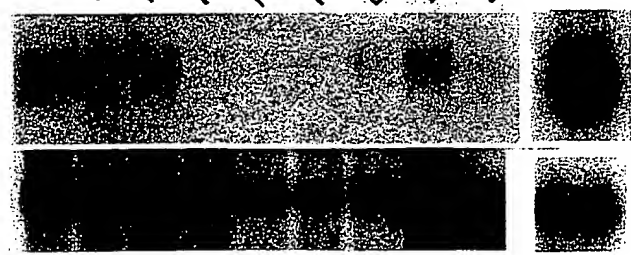
Kidney: 3E6



Placenta: 3E6

B.

Prostate  
Prostate  
Prostate  
Kidney  
Kidney  
Kidney  
Bladder  
Bladder  
Bladder  
LAPC 9

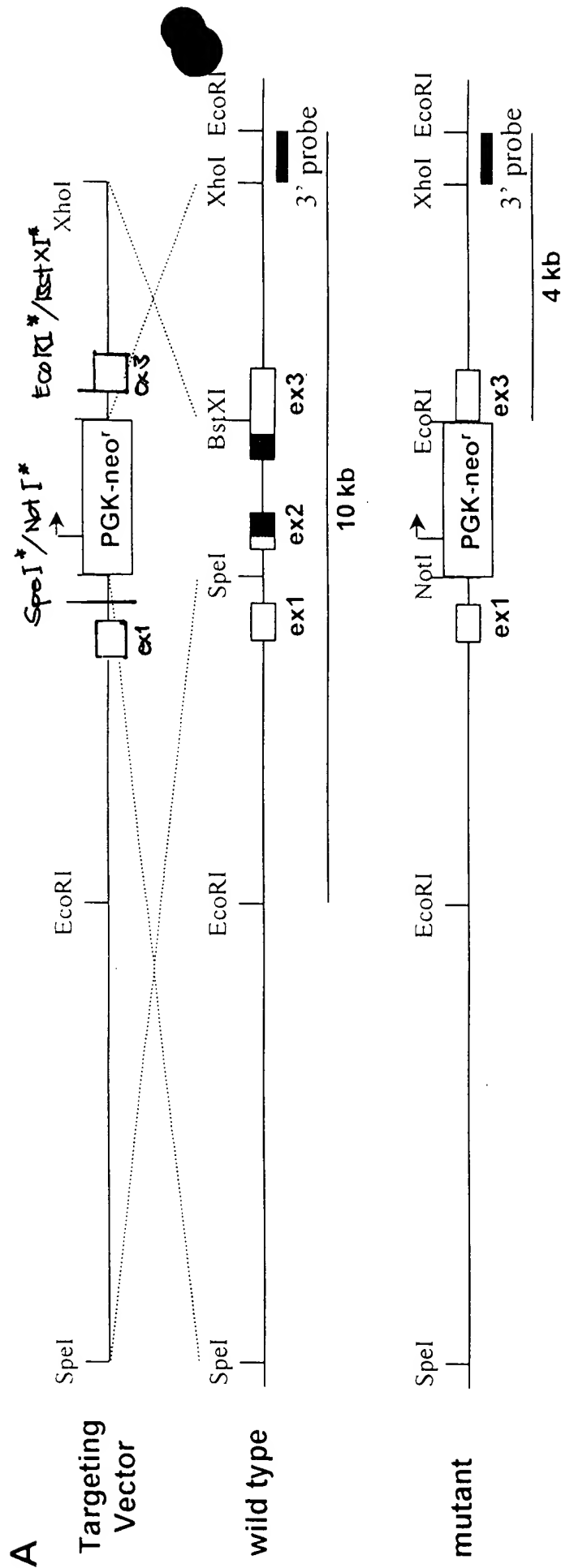


PSCA

Actin

FIGURE 39

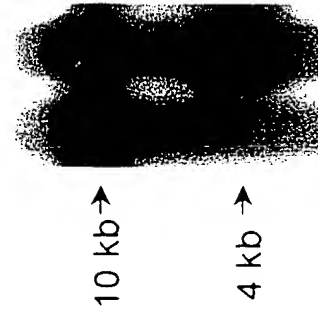
# Targeting of Mouse PSCA Gene



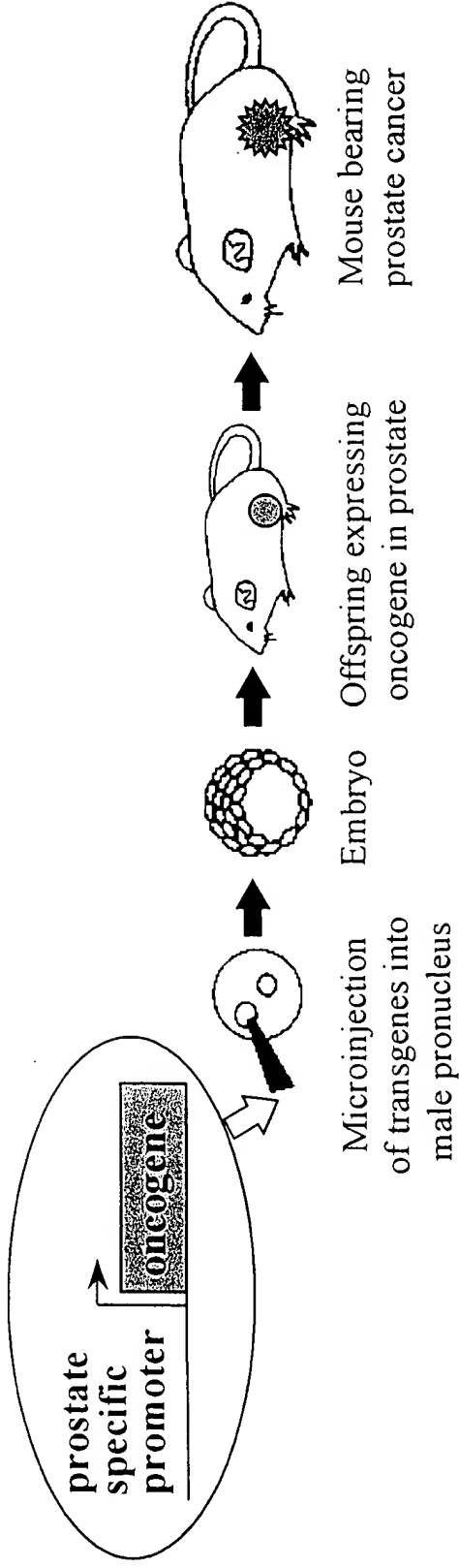
## B. Genomic Southern Analysis of ES Cells

- \* ex1, 2, and 3 are the exons of PSCA gene.
- \* Black boxes of ex2 and ex3 encode PSCA mature protein sequences.
- \* ES genomic DNAs were digested with EcoRI, followed by Southern hybridization using 3' probe.

+/- +/-



# Transgenic Mouse Models of Prostate Cancer



Transgene	Target tissues	Characteristics
C3(1) (-3 kb)/ SV40 large+small, T <i>Maroulakou et al.</i> 1994 <i>PNAS</i>	prostate (secretory cells) urethral, mammary and sweat gland	Low-grade PIN 8-12 wks High-grade PIN 8-12 wks Invasive carcinoma 28 wks No metastases
Probasin (-426 bp)/ SV40 large+small, T <i>Greenberg et al.</i> 1995 <i>PNAS</i>	prostate (secretory cells)	Low-grade PIN 5-8 wks High-grade PIN 8-12 wks Invasive carcinoma 12 wks Metastases in lymph node, lung, liver and bone
Cryptdin2 (-6.5 kb)/ SV40 large+small, T <i>Garabedian et al.</i> 1998 <i>PNAS</i>	prostate (neuroendocrine cells) small intestine	Low-grade PIN 8-12 wks High-grade PIN 8-12 wks Invasive carcinoma 16 wks Metastases in lymph node, lung, liver and bone

# Reporter Gene Constructs for Transfection Assay

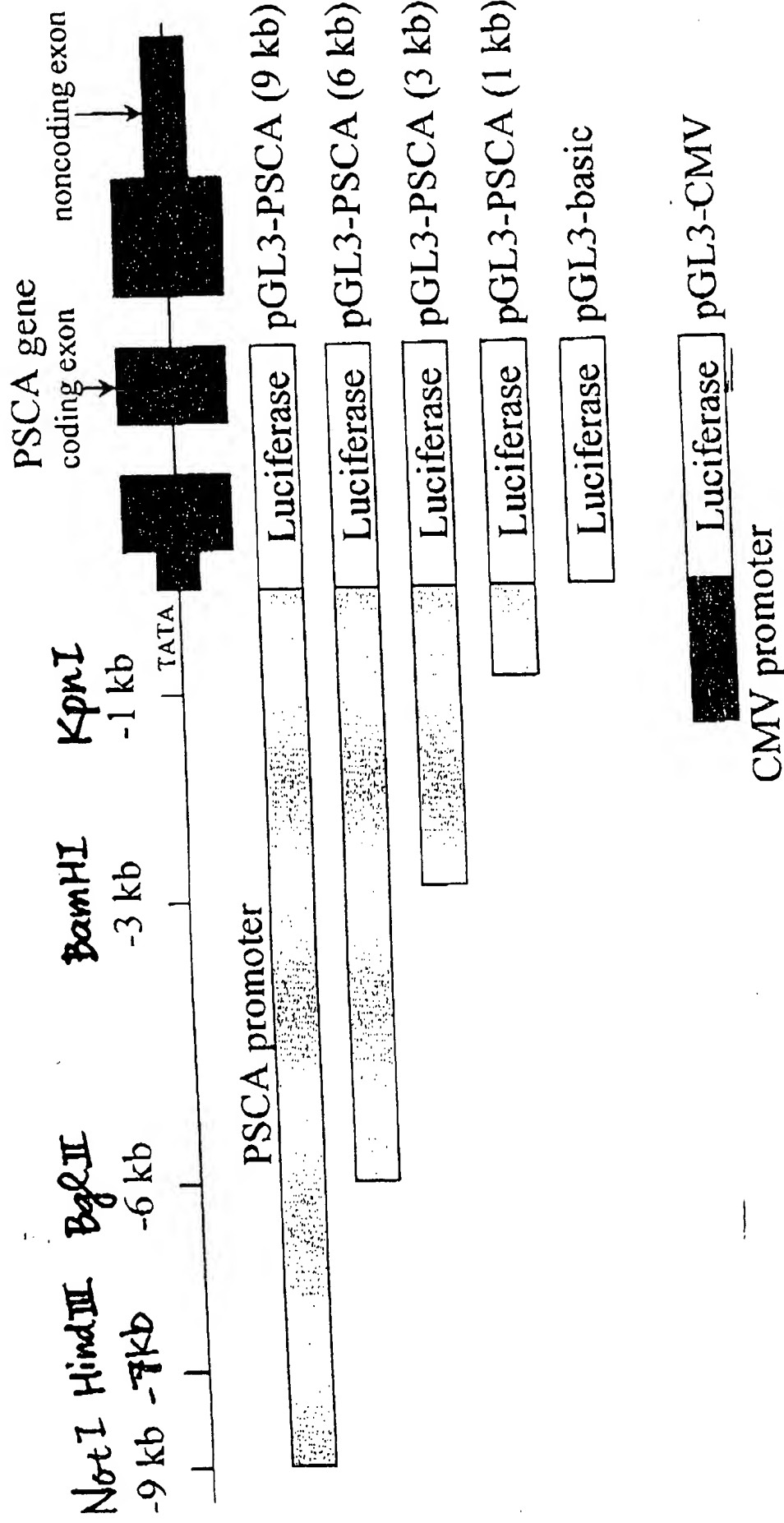


FIGURE 42



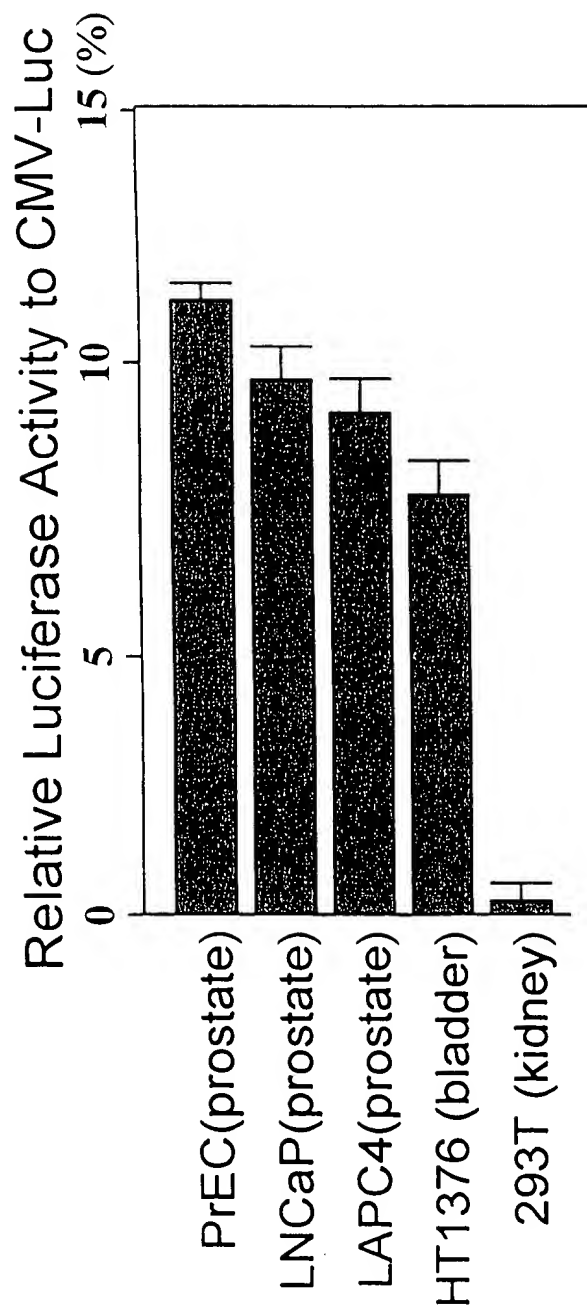


FIGURE 43

# Identification of Prostate-Specific Elements Within PSCA Promoter Sequences

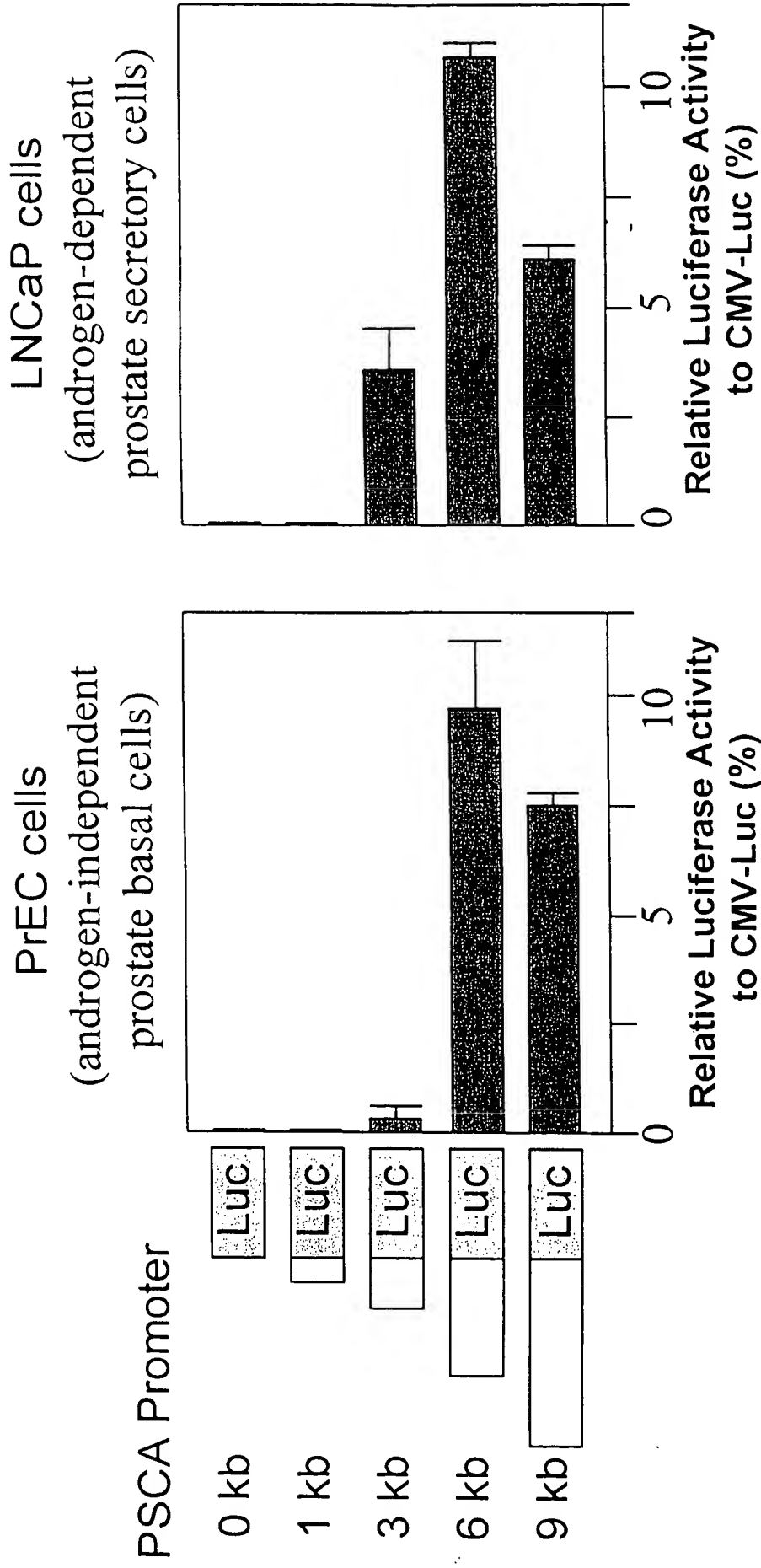


FIGURE 44

# Update of Transgenic Mouse Projects

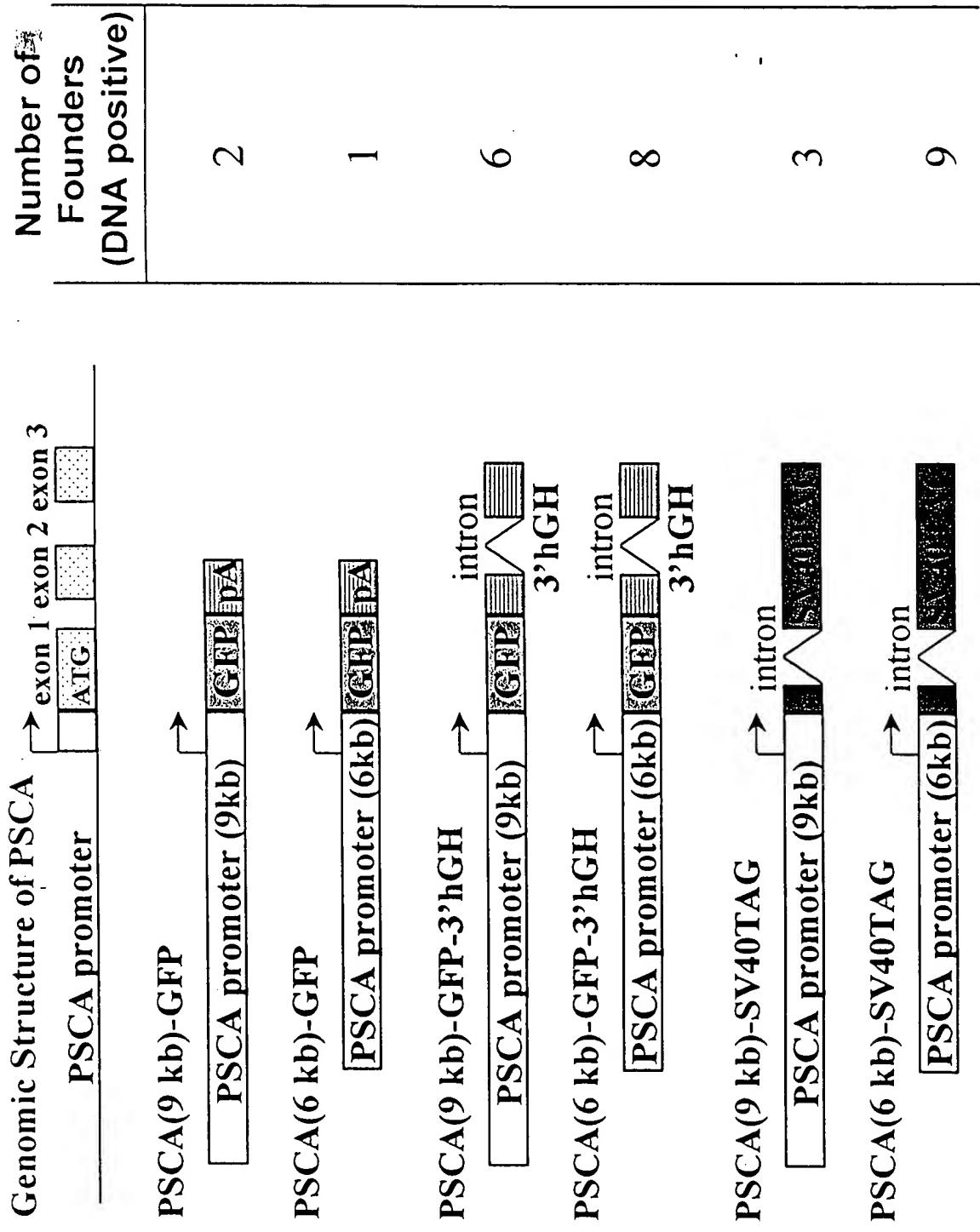


FIGURE 45

Whole-mount green fluorescence image

Negative tissues

- Stomach
- Small intestine
- Colon
- Seminal Vesicle
- Urethra
- Testis
- Liver
- Kidney
- Lung
- Brain
- Heart
- Skeletal muscle
- Ovary
- Uterus

Prostate  
(A25-106-2)

Bladder  
(A25-104)

Skin  
(A25-106-2)

Transgenic

Non-transgenic

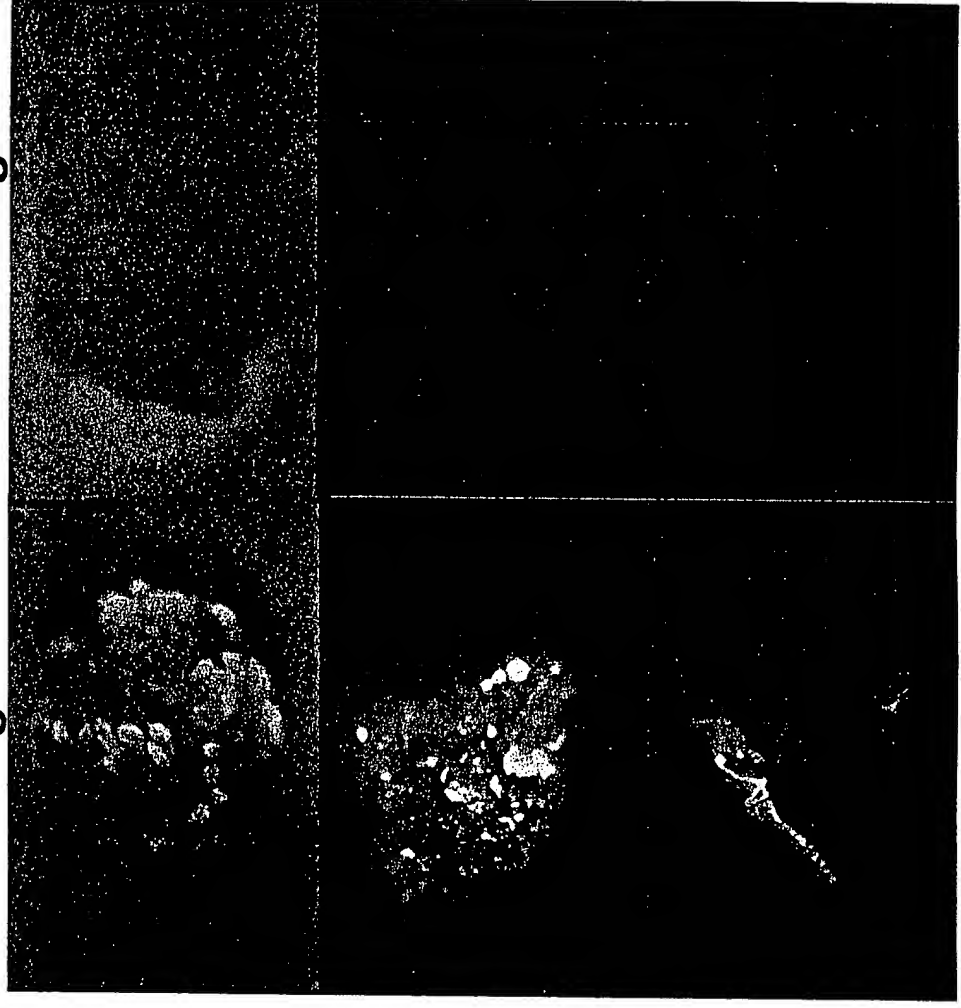


FIGURE 46

550020 32E65E60

HUMAN

Spleen  
Thymus  
Prostate  
Testis  
Ovary  
S. int.  
Colon  
PBL

Heart  
Brain  
Placenta  
Lung  
Liver  
Muscle  
Kidney  
Panc.

hPSCA →

Northern Analysis

MOUSE

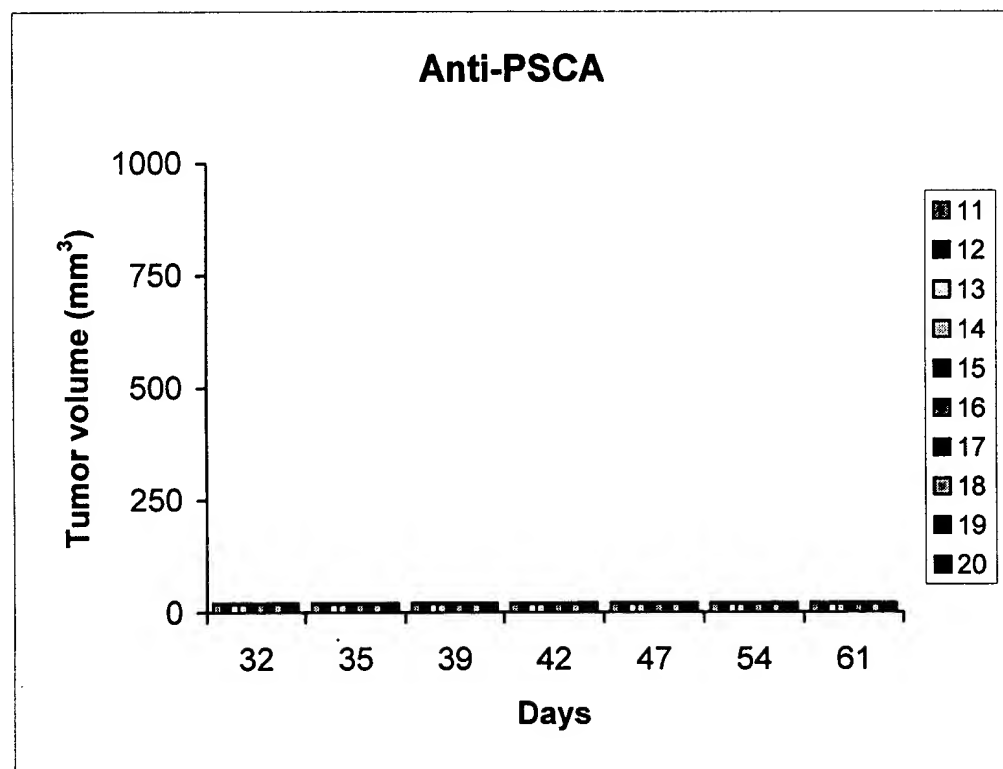
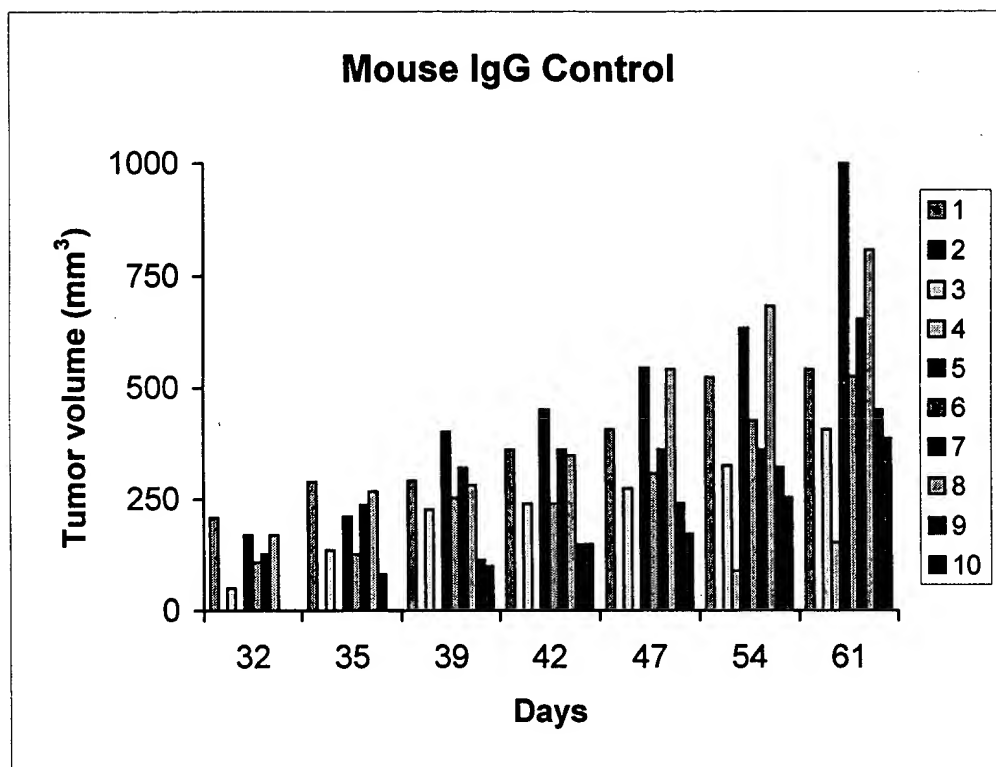
Ant. prostate  
Dorso/Lat. prostate  
Ventral prostate  
Bladder  
Seminal vesicle  
Urethra  
Testis  
Kidney  
Esophagus  
Cardiac stomach  
Body of stomach  
Pyloric stomach  
Duodenum  
Small intestine  
Colon  
Salivary gland  
Spleen  
Thymus  
Bone marrow  
Skeletal muscle  
Heart  
Brain  
Eye  
Lung  
Liver  
Skin

mPSCA →

mG3PDH →

RT-PCR

FIGURE 47



A

Epitope recognized (OD 450 nm)

mAb	Isotype	F (18-98)	N (2-50)	M (46-109)	C (85-123)
1G8	IgG1 k	1.485	0.004	1.273	0.003
2A2	IgG2a k	0.973	0.631	0.023	0.010
2H9	IgG1 k	1.069	1.026	0.002	0.001
3C5	IgG2a k	1.916	1.709	0.006	0.002
3E6	IgG3 k	1.609	0.036	1.133	2.118
3G3	IgG2a k	2.805	1.731	0.004	0.000
4A10	IgG2a k	1.053	0.493	0.000	0.001

B

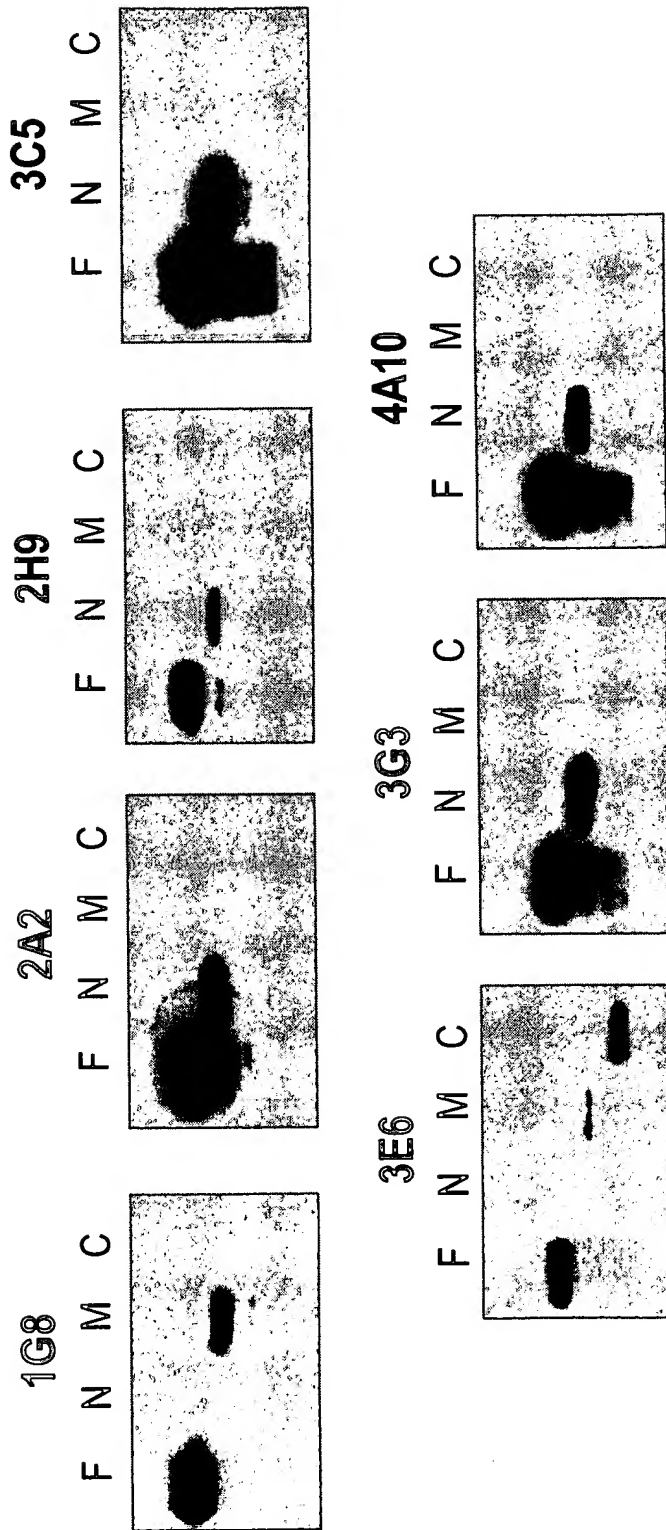
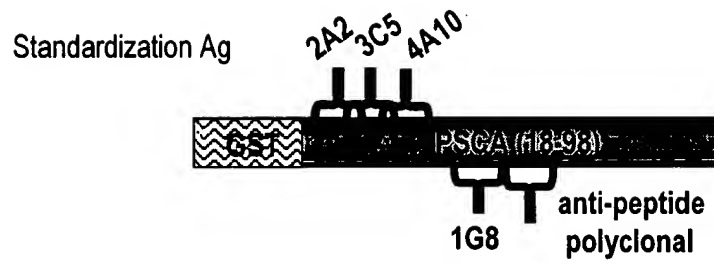


FIG. 50

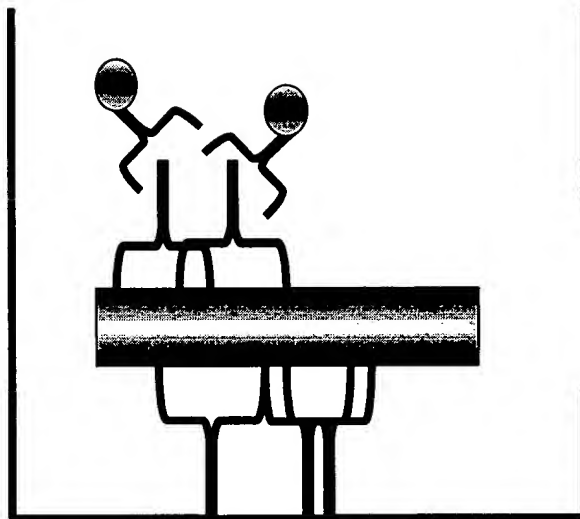
A



Engineered mammalian secreted form



B



Anti-IgG2a HRP

Anti-PSCA mAbs 3C5+4A10+2A2 (IgG2a)

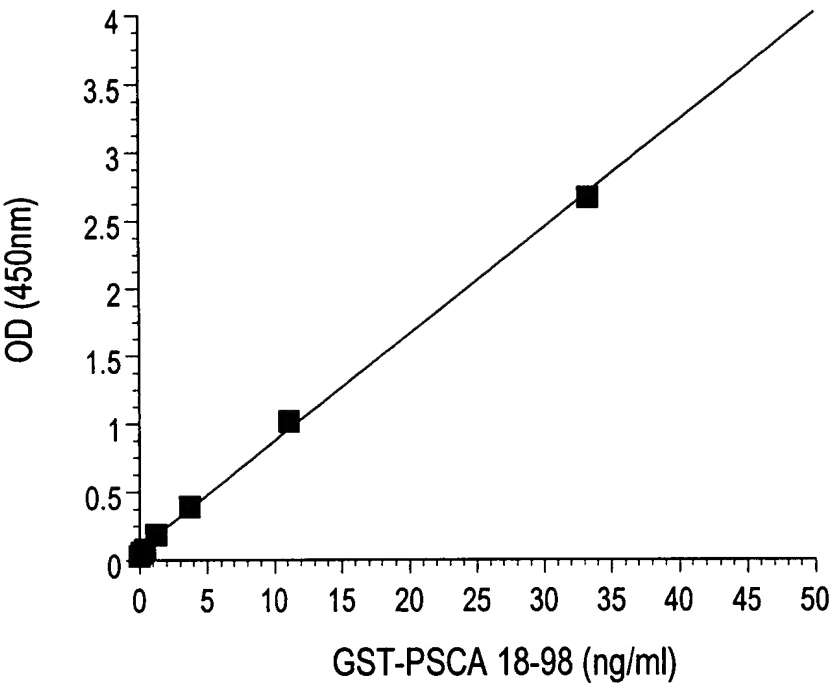
PSCA

Affinity purified anti-peptide polyclonal  
+ mAb 1G8 (IgG1)



FIG. 51

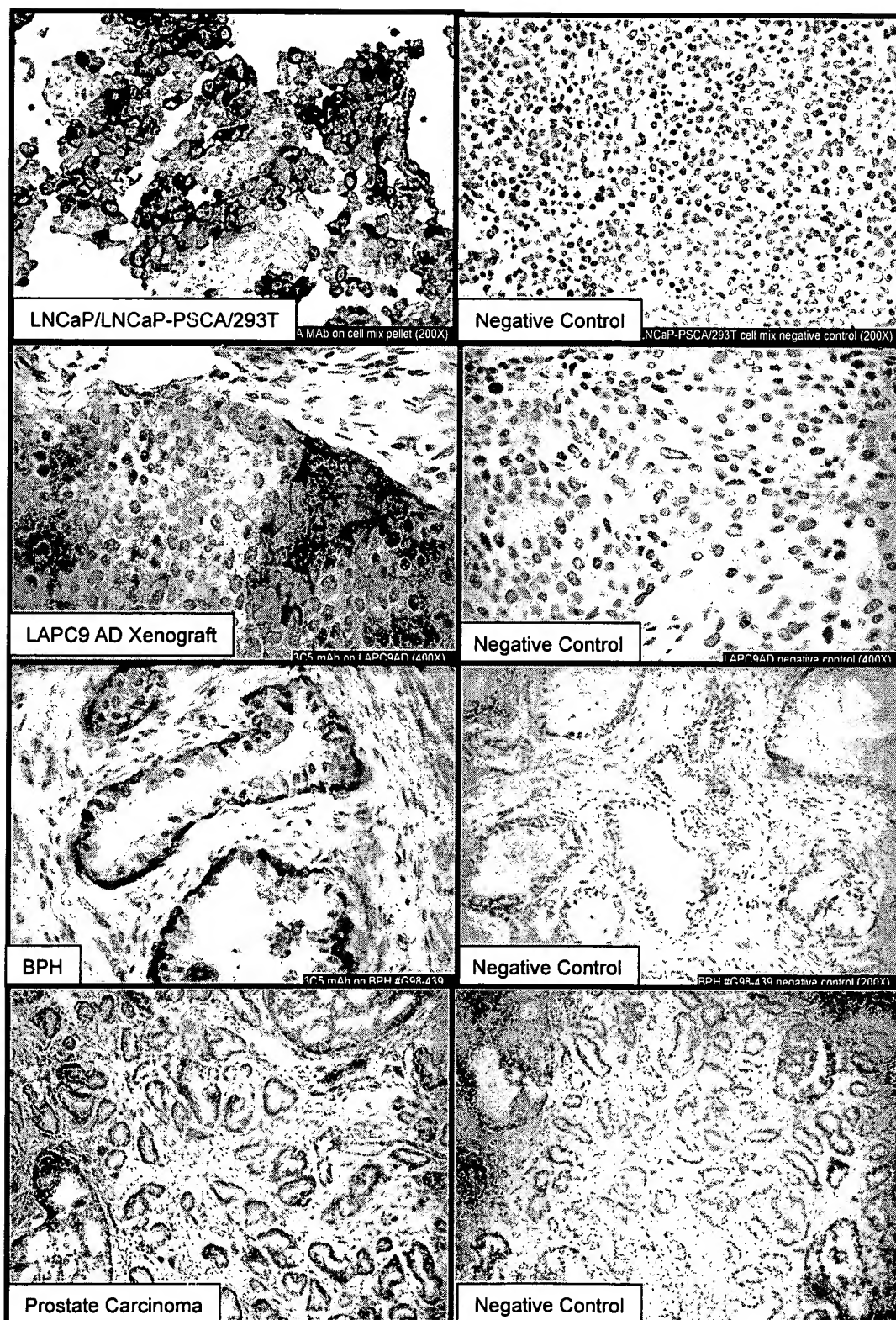
A



B

Sample	OD+range (n=2)	ng/ml
vector	0.005+0.001	ND
vector+hu serum	0.004+0.001	ND
secPSCA	2.695+0.031	32.92
secPSCA+hu serum	2.187+0.029	26.55

FIG. 52



662620 926366

FIG. 53

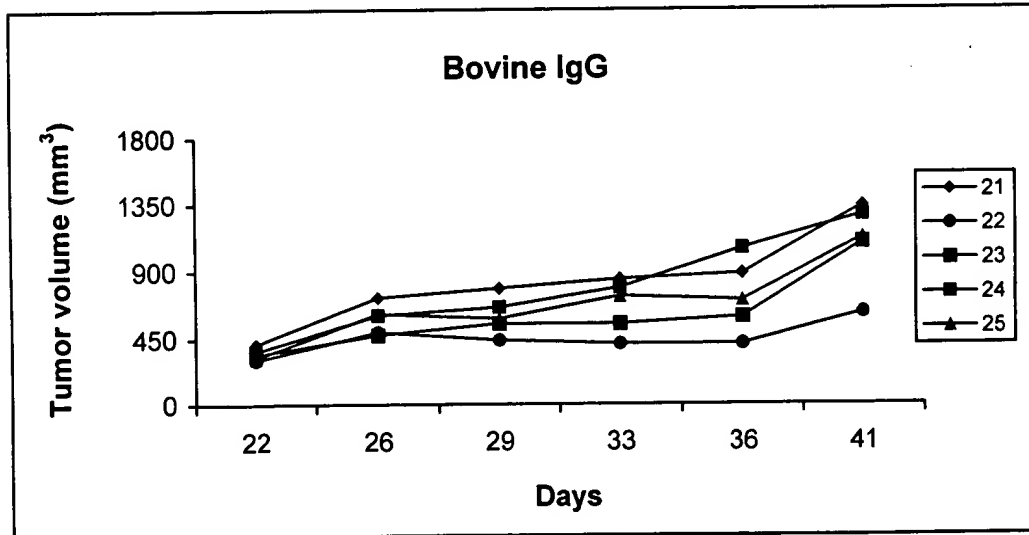
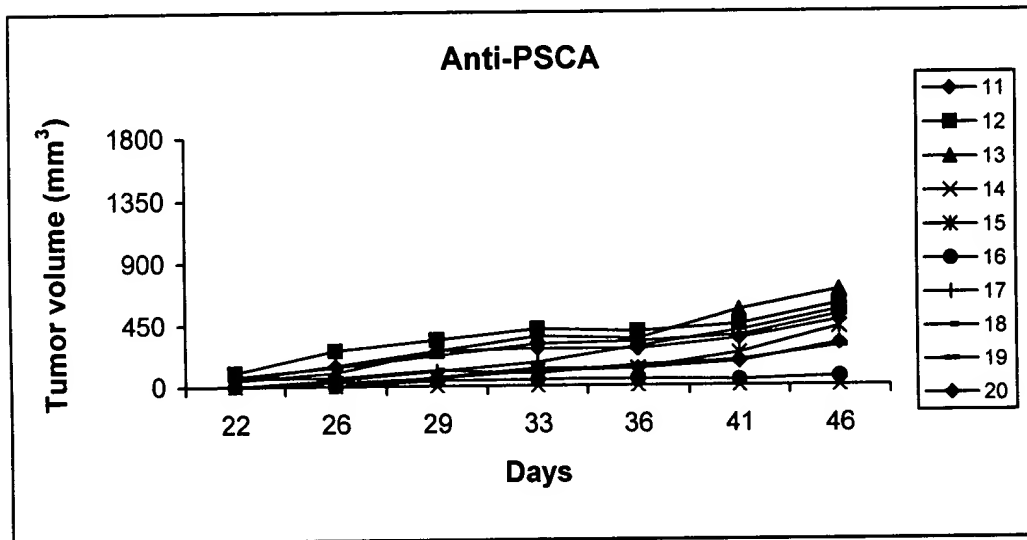
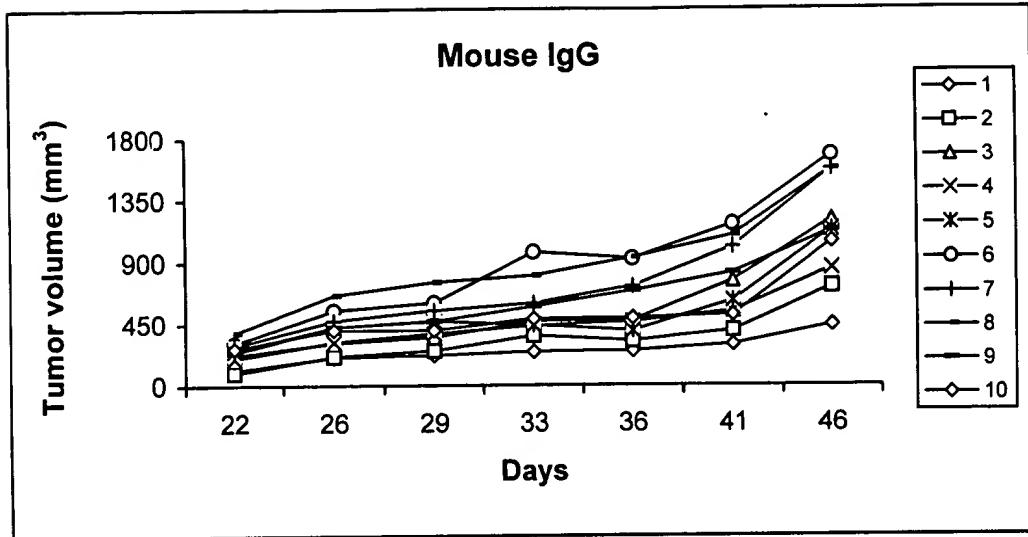


FIG. 54

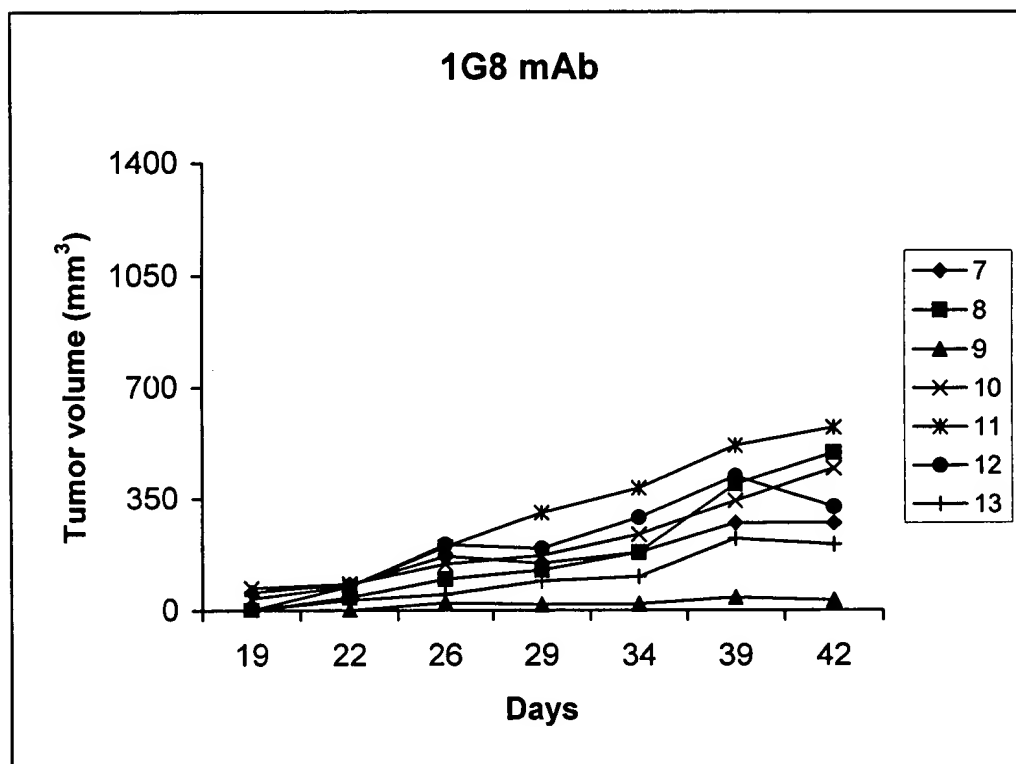
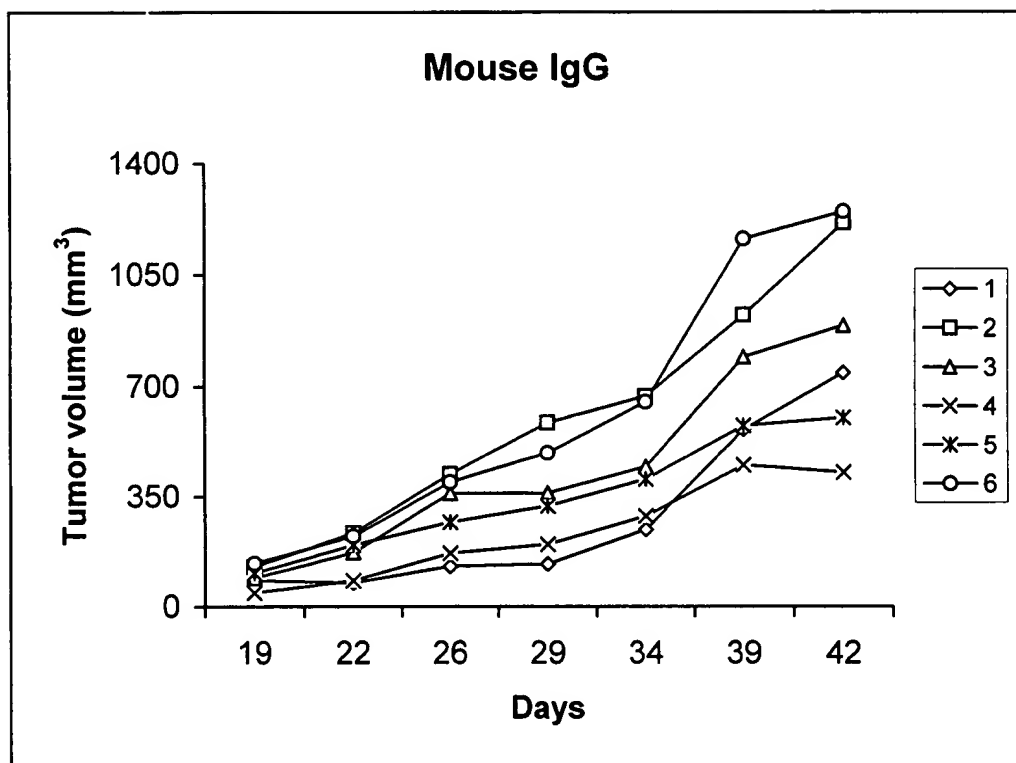


FIG. 55

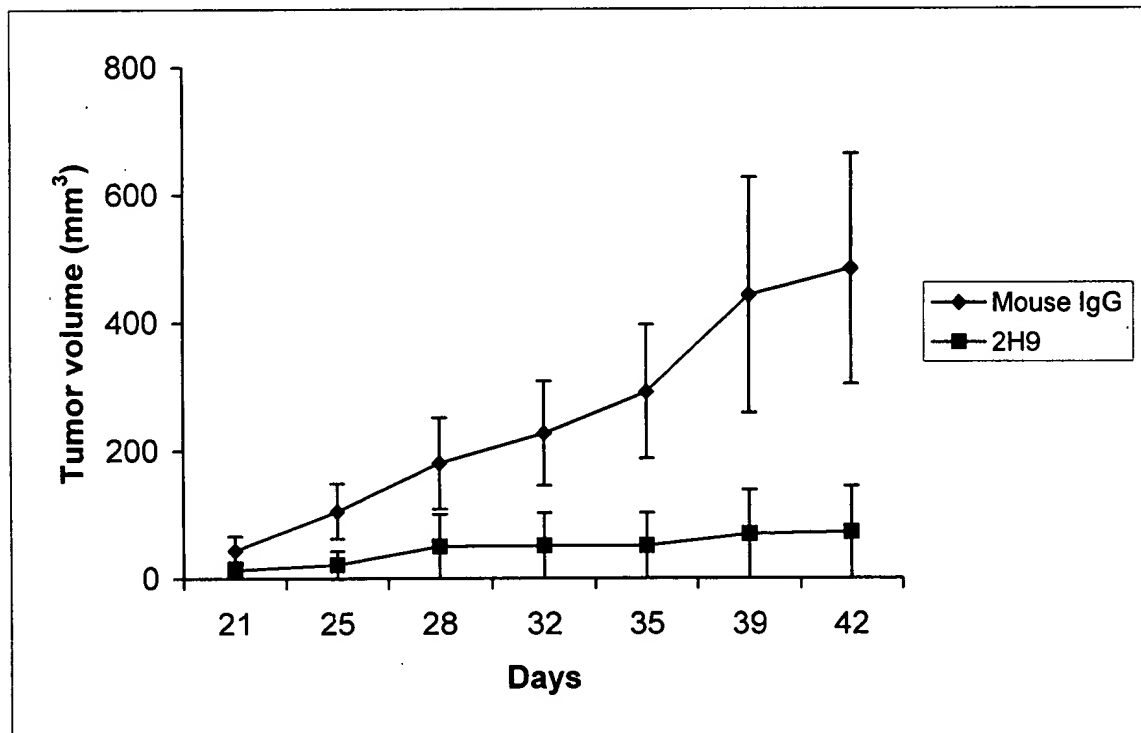
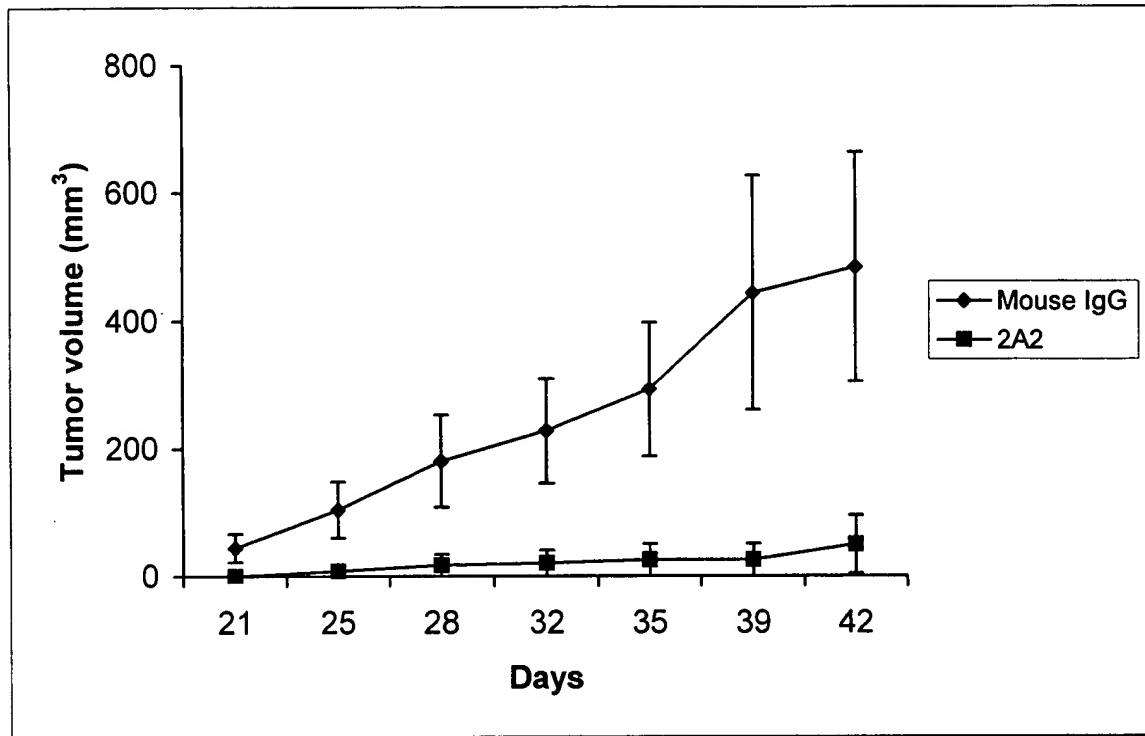


FIG. 56

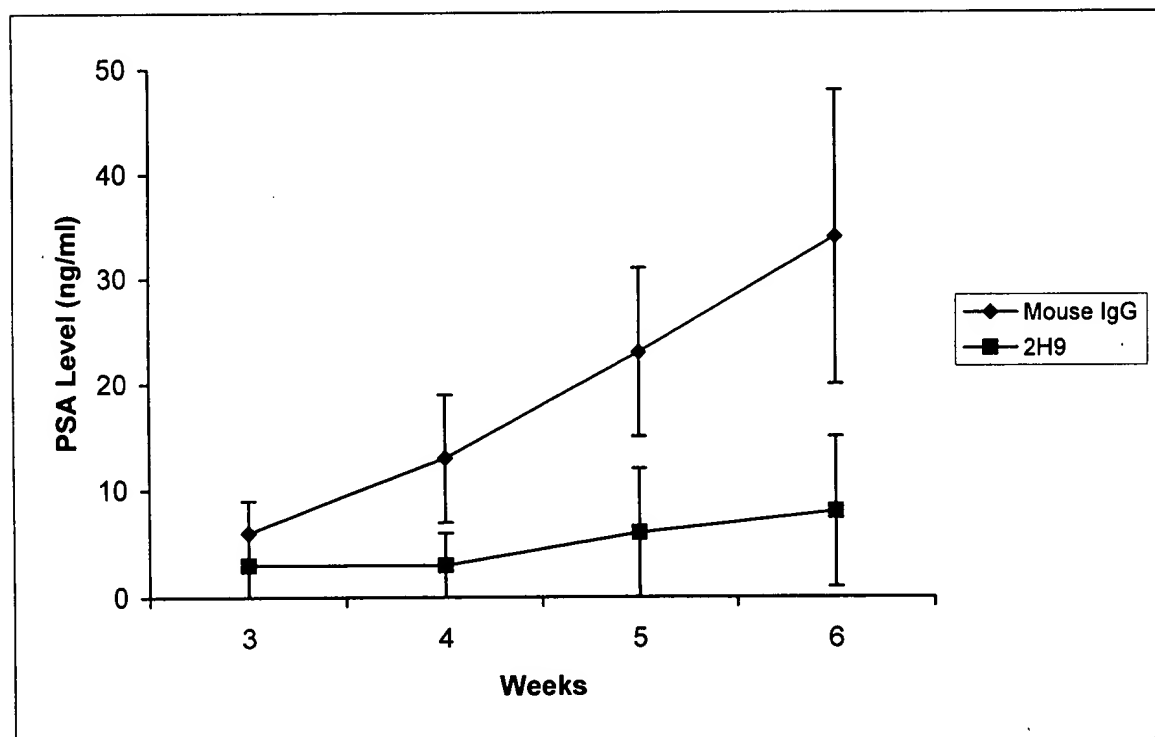
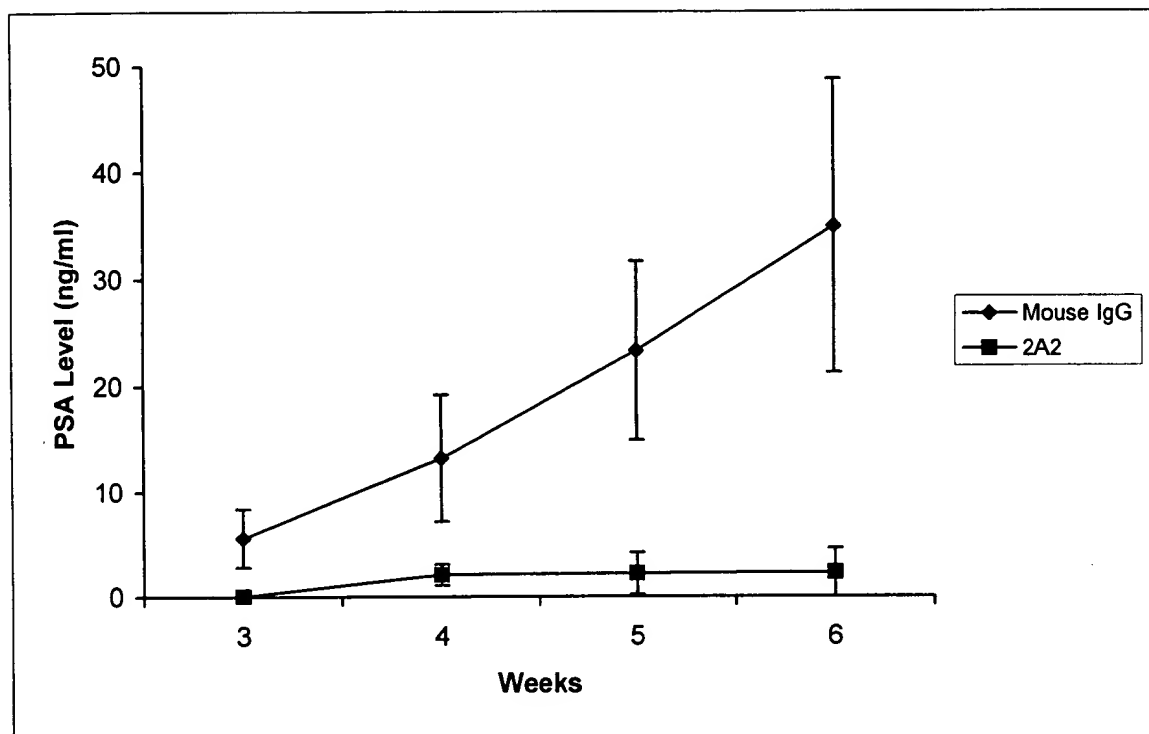


FIG. 57

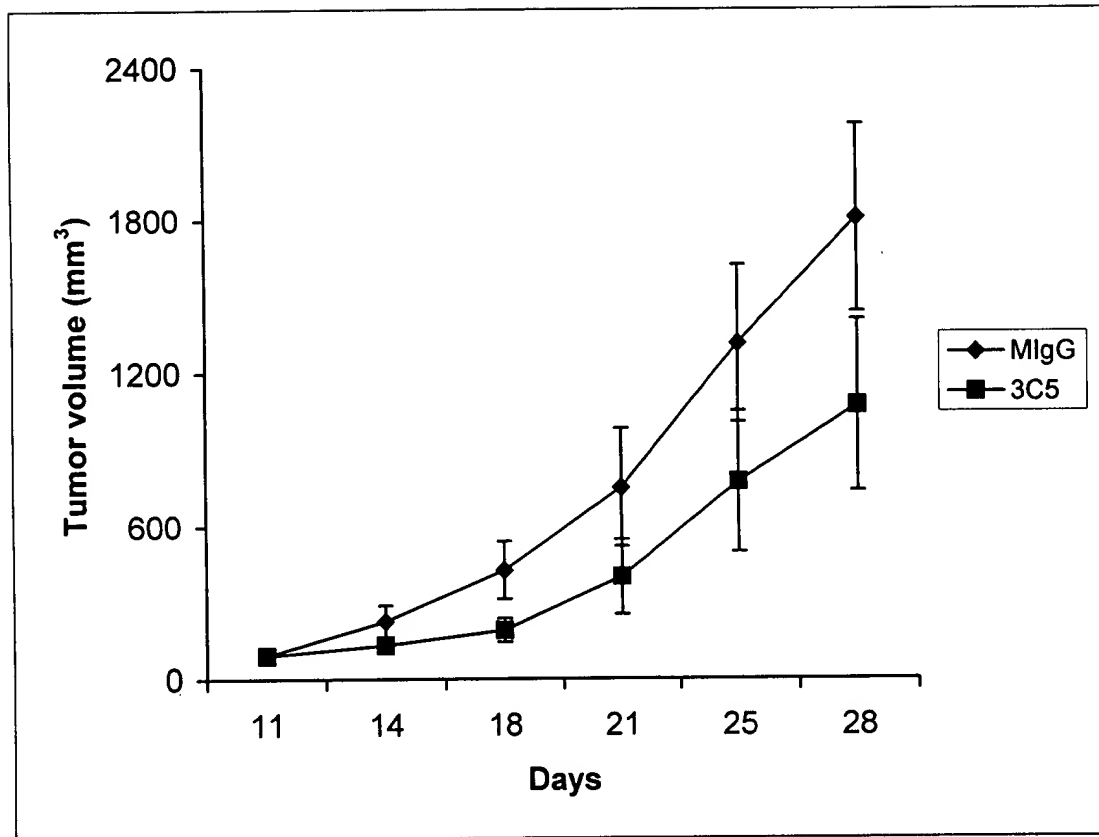


FIG. 58

TGCTTCTTCCTGATGGCAGTGGTTATAGGAGTCAATTCAGAGGTTTCAGCTGCAGCAGTCT 60  
C F F L M A V V I G V N S E V Q L Q Q S 20

GGGGCAGAACTTGTGAGGTCAGGGGCCTCAGTCAAGTTGTCCTGCACAGCTTCTGGCTTC 120  
G A E L V R S G A S V K L S C T A S G F 40

———— CDR1 —————  
AACATTAAAGACTACTATATACACTGGGTGAATCAGAGGCCTGACCAGGGCCTGGAGTGG 180  
N I K D Y Y I H W V N Q R P D Q G L E W 60

———— CDR2 —————  
ATTGGATGGATTGATCCTGAGAATGGTGACACTGAATTTGTCCCGAAGTTCCAGGGCAAG 240  
I G W I D P E N G D T E F V P K F O G K 80

GCCACTATGACTGCAGACATTTTCTCCAACACAGCCTACCTGCACCTCAGCAGCCTGACA 300  
A T M T A D I F S N T A Y L H L S S L T 100

———— CDR3 ————  
TCTGAAGACACTGCCGTCTATTACTGTAAAACGGGGGGTTTCTGGGGCCAAGGGACTCTG 360  
S E D T A V Y Y C K T G G F W G Q G T L 120

GTCACTGTCTCTGCAGCCAAAACGACACCCCCATCTGTCTATCCACTG  
V T V S A A K T T P P S V Y P L

66020"366260



FIG. 59

TTGGTAGCAACAGCCTCAGATGTCCACTCCCAGGTCCAAGTGCAGCAACCTGGGTCTGAA 60  
L V A T A S D V H S Q V Q L Q Q P G S E 20

CTGGTGAGGCCTGGAACCTCAGTGAAGCTGTCCTGCAAGGCTTCTGGCTATACATTCTCC 120  
L V R P G T S V K L S C K A S G Y T F S 40  
CDR1

AGCTACTGGATGCACTGGGTGAAGCAGAGGCCTGGACAAGGCCTTGAGTGGATTGGAAAT 180  
S Y W M H W V K Q R P G Q G L E W I G N 60

ATTGACCCTGGTAGTGGTTACACTAACTACGCTGAGAACCTCAAGACCAAGGCCACACTG 240  
I D P G S G Y T N Y A E N L K T K A T L 80  
CDR2

ACTGTAGACACATCCTCCAGCACAGCCTACATGCAGCTCAGCAGCCTGACATCTGAGGAC 300  
T V D T S S S T A Y M Q L S S L T S E D 100

TCTGCAGTCTATTACTGTACAAGCCGATCTACTATGATTACGACGGGATTGCTTACTGG 360  
S A V Y Y C T S R S T M I T T G F A Y W 120  
CDR3

GGCCAAGGGACTCTGGTCACTGTCTCTGCAGCTACAACAACAGCCCCATCTGTCTATCCA 420  
G Q G T L V T V S A A T T T A P S V Y P 160

CTGGCC  
L A

[illegible]

CCCCATCTGTCTATCCACTGGCCCCTTGTGTA  
P P S V Y P L A P C V



FIG. 62

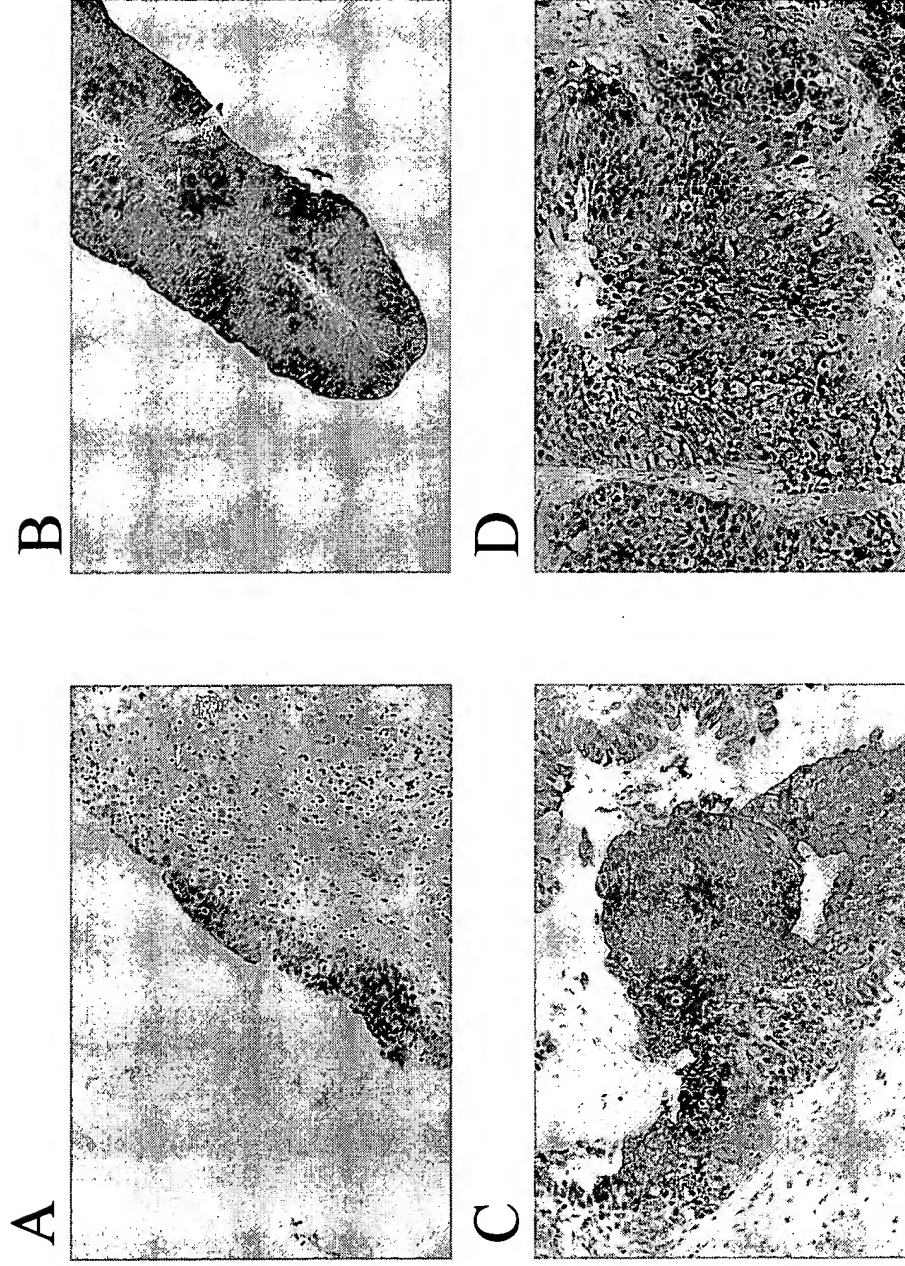


FIG. 63

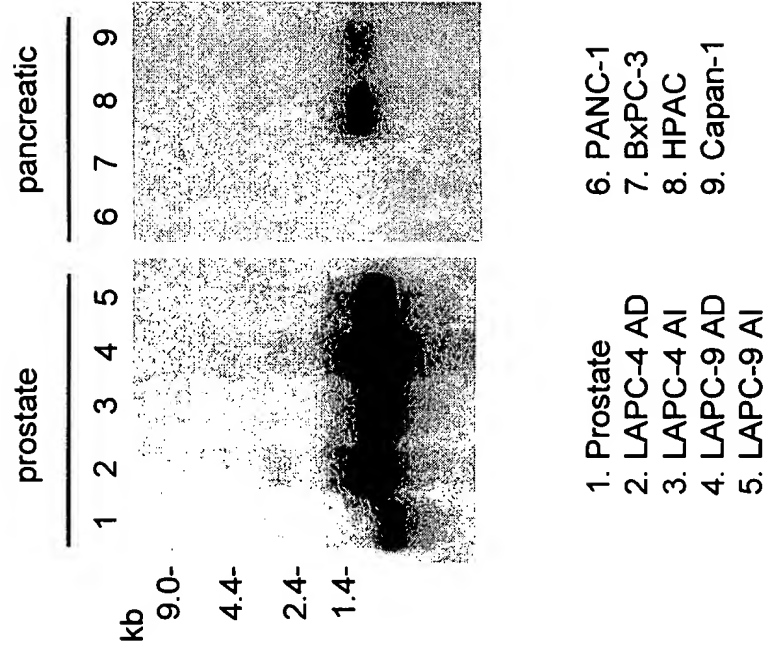
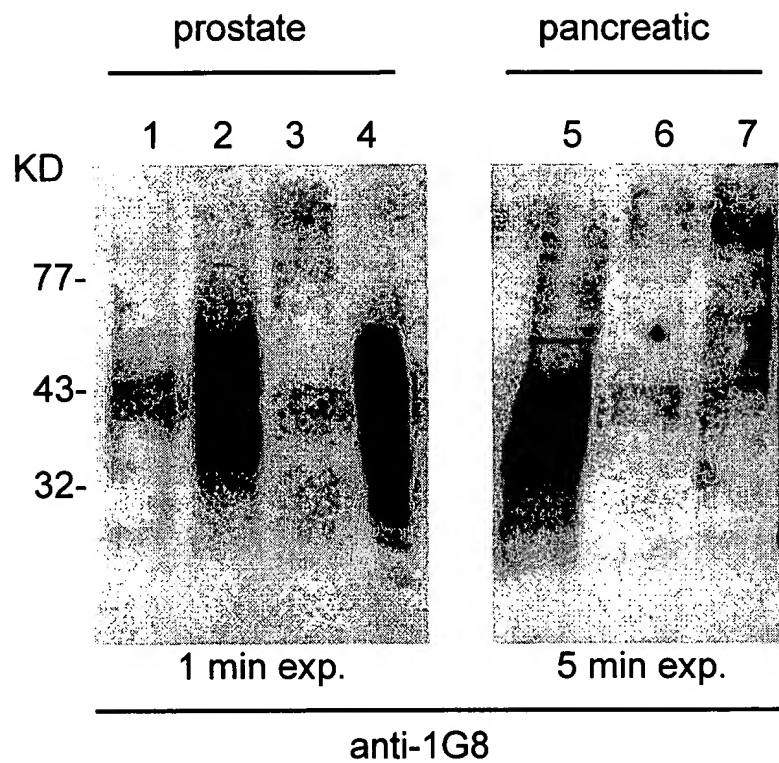


FIG. 64



1. LAPC-4 AD
2. LAPC-9 AI
3. LNCaP
4. LNCaP-PSCA

5. HPAC
6. Capan-1
7. ASPC-1

60000 30000 0

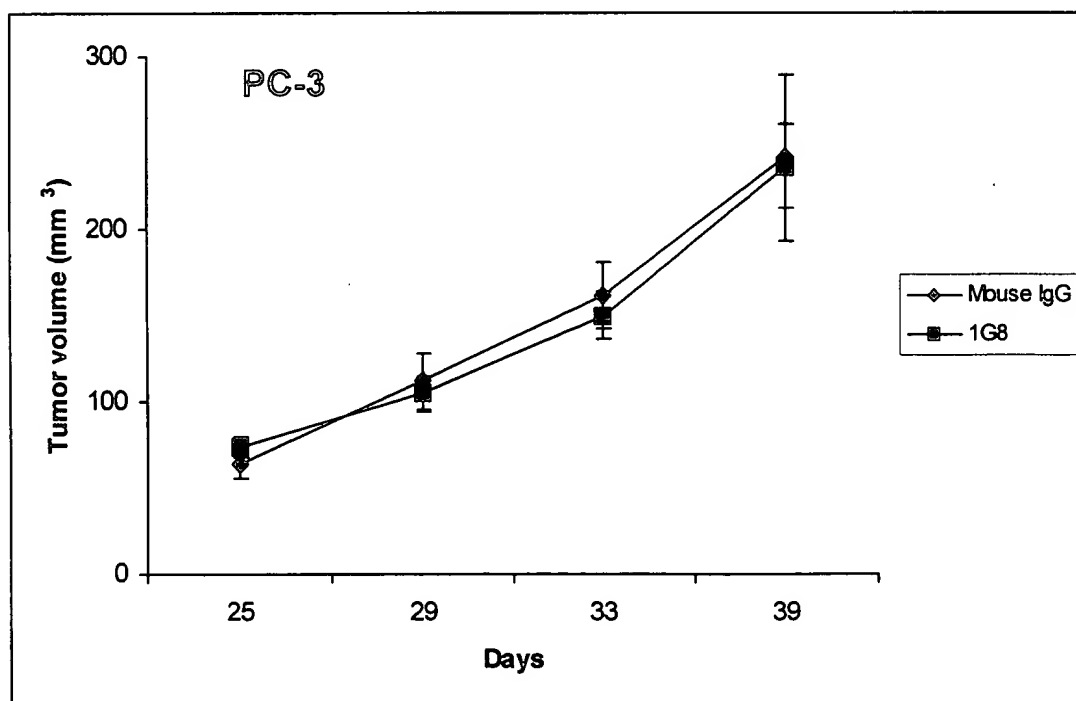
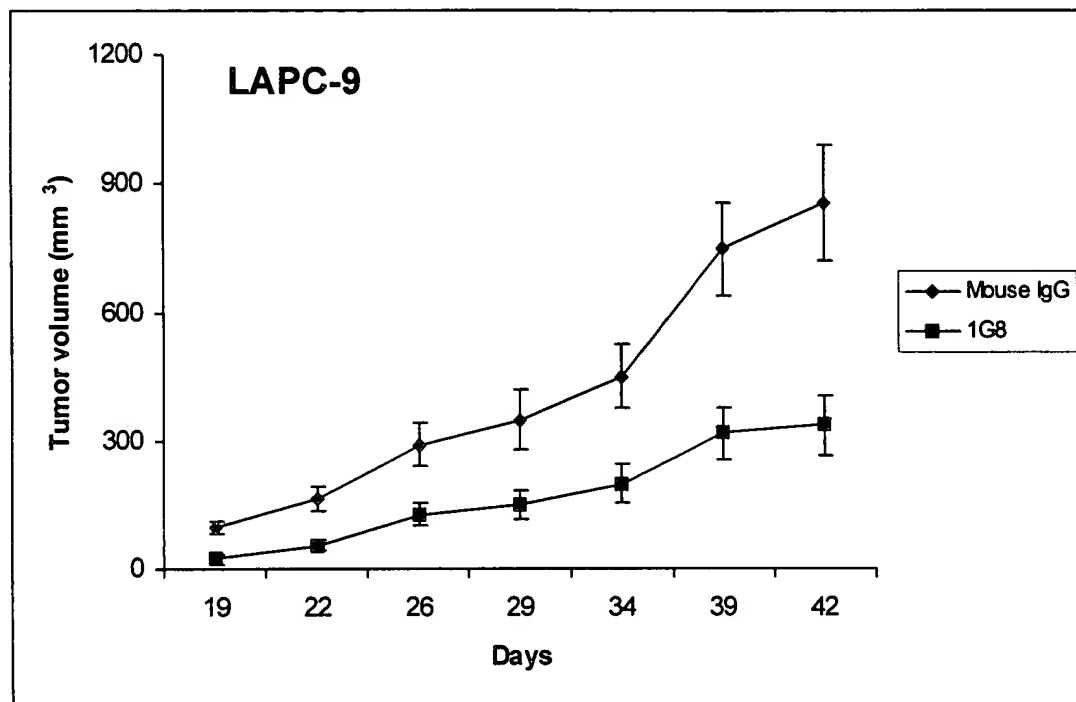


FIGURE 65